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(54) Title: FULLY HUMAN ANTIBODIES DIRECTED AGAINST THE HUMAN INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR

(57) Abstract: This invention relates to human antibodies that bind to human insulin-like growth factor-1 receptor (IGF-IR), to derivatives of these antibodies (Fabs, single chain antibodies, bi-specific antibodes, or fusion proteins), and to uses of the antibodies and derivatives in therapeutic, and diagnostic methods. The invention relates to nucleic acids encoding the anti-IGF-IR, methods of generating the antibodies and expression. The invention further relates to combination therapies using ant-IGF-IR antibodies with anti-neoplastic drugs.

FULLY HUMAN ANTIBODIES DIRECTED AGAINST THE HUMAN INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR

[0001] This application claims the benefit of United States Provisional Application 60/467,177, filed May 1, 2003.

BACKGROUND

[0002] The insulin-like growth factor receptor (IGF-IR) is a ubiquitous transmembrane tyrosine kinase receptor that is essential for normal fetal and post-natal growth and development. IGF-IR can stimulate cell proliferation, cell differentiation, changes in cell size, and protect cells from apoptosis. It has also been considered to be quasi-obligatory for cell transformation (reviewed in Adams et al., *Cell. Mol. Life Sci.* 57:1050-93 (2000); Baserga, *Oncogene* 19:5574-81 (2000)). The IGF-IR is located on the cell surface of most cell types and serves as the signaling molecule for growth factors IGF-I and IGF-II (collectively termed henceforth IGFs). IGF-IR also binds insulin, albeit at three orders of magnitude lower affinity than it binds to IGFs. IGF-IR is a pre-formed hetero-tetramer containing two alpha and two beta chains covalently linked by disulfide bonds. The receptor subunits are synthesized as part of a single polypeptide chain of 180kd, which is then proteolytically processed into alpha (130kd) and beta (95kd) subunits. The entire alpha chain is extracellular and contains the site for ligand binding. The beta chain possesses the transmembrane domain, the tyrosine kinase domain, and a C-terminal extension that is necessary for cell differentiation and transformation, but is dispensable for mitogen signaling and protection from apoptosis.

[0003] IGF-IR is highly similar to the insulin receptor (IR), particularly within the beta chain sequence (70% homology). Because of this homology, recent studies have demonstrated that these receptors can form hybrids containing one IR dimer and one IGF-IR dimer (Pandini et al., *Clin. Canc. Res.* 5:1935-19 (1999)). The formation of hybrids occurs in both normal and transformed cells and the hybrid content is dependent upon the concentration of the two homodimer receptors (IR and IGF-IR) within the cell. In one study of 39 breast cancer specimens, although both IR and IGF-IR were over-expressed in all tumor samples, hybrid receptor content consistently exceeded the levels of both homo-receptors by approximately 3-fold (Pandini et al., *Clin. Canc. Res.* 5:1935-44 (1999)). Although hybrid receptors are composed of IR and IGF-IR pairs, the hybrids bind selectively to IGFs, with

affinity similar to that of IGF-IR, and only weakly bind insulin (Siddle and Soos, The IGF System. Humana Press. pp. 199-225. 1999). These hybrids therefore can bind IGFs and transduce signals in both normal and transformed cells.

[0004] A second IGF receptor, IGF-IIR, or mannose-6-phosphate (M6P) receptor, also binds IGF-II ligand with high affinity, but lacks tyrosine kinase activity (Oates et al., *Breast Cancer Res. Treat.* 47:269-81 (1998)). Because it results in the degradation of IGF-II, it is considered a sink for IGF-II, antagonizing the growth promoting effects of this ligand. Loss of the IGF-IIR in tumor cells can enhance growth potential through release of its antagonistic effect on the binding of IGF-II with the IGF-IR (Byrd et al., *J. Biol. Chem.* 274:24408-16 (1999)).

[0005] Endocrine expression of IGF-I is regulated primarily by growth hormone and produced in the liver, but recent evidence suggests that many other tissue types are also capable of expressing IGF-I. This ligand is therefore subjected to endocrine and paracrine regulation, as well as autocrine in the case of many types of tumor cells (Yu, H. and Rohan, J., *J. Natl. Cancer Inst.* 92:1472-89 (2000)).

[0006] Six IGF binding proteins (IGFBPs) with specific binding affinities for the IGFs have been identified in serum (Yu, H. and Rohan, J., *J. Natl. Cancer Inst.* 92:1472-89 (2000)). IGFBPs can either enhance or inhibit the action of IGFs, as determined by the molecular structures of the binding proteins as a result of post-translational modifications. Their primary roles are for transport of IGFs, protection of IGFs from proteolytic degradation, and regulation of the interaction of IGFs with IGF-IR. Only about 1% of serum IGF-I is present as free ligand, the remainder is associated with IGFBPs (Yu, H. and Rohan, J., *J. Natl. Cancer Inst.* 92:1472-89 (2000)).

[0007] Upon binding of ligand (IGFs), the IGF-IR undergoes autophosphorylation at conserved tyrosine residues within the catalytic domain of the beta chain. Subsequent phosphorylation of additional tyrosine residues within the beta chain provides docking sites for the recruitment of downstream molecules critical to the signaling cascade. The principle pathways for transduction of the IGF signal are mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) (reviewed in Blakesley et al., In: The IGF System. Humana Press. 143-163 (1999)). The MAPK pathway is primarily responsible for the mitogenic signal elicited following IGFs stimulation and PI3K is responsible for the IGF-dependent induction of anti-apoptotic or survival processes.

[0008] A key role of IGF-IR signaling is its anti-apoptotic or survival function. Activated IGF-IR signals PI3K and downstream phosphorylation of Akt, or protein kinase B. Akt can effectively block, through phosphorylation, molecules such as BAD, which are essential for the initiation of programmed cell death, and inhibit initiation of apoptosis (Datta et al., *Cell* 91:231-41 (1997)). Apoptosis is an important cellular mechanism that is critical to normal developmental processes (Oppenheim, *Annu. Rev. Neurosci.* 14:453-501 (1991)). It is a key mechanism for effecting the elimination of severely damaged cells and reducing the potential persistence of mutagenic lesions that may promote tumorigenesis. To this end, it has been demonstrated that activation of IGFs signaling can promote the formation of spontaneous tumors in a mouse transgenic model (DiGiovanni et al., *Cancer Res.* 60:1561-70 (2000)). Furthermore, IGF over-expression can rescue cells from chemotherapy induced cell death and may be an important factor in tumor cell drug resistance (Gooch et al., *Breast Cancer Res. Treat.* 56:1-10 (1999)). Consequently, modulation of the IGF signaling pathway has been shown to increase the sensitivity of tumor cells to chemotherapeutic agents (Benini et al., *Clinical Cancer Res.* 7:1790-97 (2001)).

[0009] A large number of research and clinical studies have implicated the IGF-IR and its ligands (IGFs) in the development, maintenance, and progression of cancer. In tumor cells, over-expression of the receptor, often in concert with over-expression of IGF ligands, leads to potentiation of these signals and, as a result, enhanced cell proliferation and survival. IGF-I and IGF-II have been shown to be strong mitogens for a wide variety of cancer cell lines including prostate (Nickerson et al., *Cancer Res.* 61:6276-80 (2001); Hellawell et al., *Cancer Res.* 62:2942-50 (2002)) breast (Gooch et al., *Breast Cancer Res. Treat.* 56:1-10 (1999)), lung, colon (Hassan and Macaulay, *Ann. Oncol.* 13:349-56 (2002)), stomach, leukemia, pancreas, brain, myeloma (Ge and Rudikoff, *Blood* 96:2856-61 (2000), melanoma (All-Ericsson et al., *Invest. Ophthalmol. Vis. Sci.* 43:1-8 (2002)), and ovary (reviewed in: Macaulay, *Br. J. Cancer* 65:311-20 (1990)) and this effect is mediated through the IGF-IR. High circulating levels of IGF-I in serum have been associated with an increased risk of breast, prostate, and colon cancer (Pollak, *Eur. J. Cancer* 36:1224-28 (2000)). In a mouse model of colon cancer, increases in circulating IGF-I levels *in vivo* led to a significant increase in the incidence of tumor growth and metastasis (Wu et al., *Cancer Res.* 62: 1030-35 (2002)). Constitutive expression of IGF-I in epidermal basal cells of transgenic mice has been shown to promote spontaneous tumor formation (DiGiovanni et al., *Cancer Res.*

60:1561-1570 (2000; Bol et al., *Oncogene* 14:1725-1734 (1997)). Over-expression of IGF-II in cell lines and tumors occurs with high frequency and may result from loss of genomic imprinting of the IGF-II gene (Yaginuma et al., *Oncology* 54:502-7 (1997)). Receptor over-expression has been demonstrated in many diverse human tumor types including lung (Quinn et al., *J. Biol. Chem.* 271:11477-83 (1996)), breast (Cullen et al., *Cancer Res.* 50: 48-53 (1990); Peyrat and Bonneterre, *Cancer Res.* 22:59-67 (1992); Lee and Yee, *Biomed. Pharmacother.* 49:415-21 (1995)), sarcoma (van Valen et al., *J. Cancer Res. Clin. Oncol.* 118:269-75 (1992); Scotlandi et al., *Cancer Res.* 56:4570-74 (1996)), prostate (Nickerson et al., *Cancer Res.* 61:6276-80 (2001)), and colon (Hassan and Macaulay, *Ann. Oncol.* 13:349-56 (2002)). In addition, highly metastatic cancer cells have been shown to possess higher expression of IGF-II and IGF-IR than tumor cells that are less prone to metastasize (Guerra et al., *Int. J. Cancer* 65:812-20 (1996)). A critical role of the IGF-IR in cell proliferation and transformation was demonstrated in experiments of IGF-IR knockout derived mouse embryo fibroblasts. These primary cells grow at significantly reduced rates in culture medium containing 10% serum and fail to transform by a variety of oncogenes including SV40 Large T (Sell et al., *Mol. Cell. Biol.* 3604-12 (1994)). Recently it was demonstrated that resistance to the drug Herceptin in some forms of breast cancer may be due to activation of IGF-IR signaling in those cancers (Lu et al., *J. Natl. Cancer Inst.* 93:1852-57 (2001)). Over-expression or activation of IGF-IR may therefore not only be a major determinant in tumorigenicity, but also in tumor cell drug resistance.

[0010] Activation of the IGF system has also been implicated in several pathological conditions besides cancer, including acromegaly (Drange and Melmed. In: The IGF System. Humana Press. 699-720 (1999)), retinal neovascularization (Smith et al., *Nature Med.* 12:1390-95 (1999)), and psoriasis (Wraight et al., *Nature Biotech.* 18:521-26 (2000)). In the latter study, an antisense oligonucleotide preparation targeting the IGF-IR was effective in significantly inhibiting the hyperproliferation of epidermal cells in human psoriatic skin grafts in a mouse model, suggesting that anti-IGF-IR therapies may be an effective treatment for this chronic disorder.

[0011] A variety of strategies have been developed to inhibit the IGF-IR signaling pathway in cells. Antisense oligonucleotides have been effective *in vitro* and in experimental mouse models, as shown above for psoriasis. In addition, inhibitory peptides targeting the IGF-IR have been generated that possess anti-proliferative activity *in vitro* and *in vivo*

(Pietrzkowski et al., *Cancer Res.* 52:6447–51 (1992); Haylor et al., *J. Am. Soc. Nephrol.* 11:2027-35 (2000)). A synthetic peptide sequence from the C-terminus of IGF-IR has been shown to induce apoptosis and significantly inhibit tumor growth (Reiss et al., *J. Cell. Phys.* 181:124-35 (1999)). Several dominant-negative mutants of the IGF-IR have also been generated which, upon over-expression in tumor cell lines, compete with wild-type IGF-IR for ligand and effectively inhibit tumor cell growth *in vitro* and *in vivo* (Scotlandi et al., *Int. J. Cancer* 101:11-6 (2002); Seely et al., *BMC Cancer* 2:15 (2002)). Additionally, a soluble form of the IGF-IR has also been demonstrated to inhibit tumor growth *in vivo* (D'Ambrosio et al., *Cancer Res.* 56:4013-20 (1996)). Antibodies directed against the human IGF-IR have also been shown to inhibit tumor cell proliferation *in vitro* and tumorigenesis *in vivo* including cell lines derived from breast cancer (Artega and Osborne, *Cancer Res.* 49:6237-41 (1989)), Ewing's osteosarcoma (Scotlandi et al., *Cancer Res.* 58:4127-31 (1998)), and melanoma (Furlanetto et al., *Cancer Res.* 53:2522-26 (1993)). Antibodies are attractive therapeutics chiefly because of they 1) can possess high selectivity for a particular protein antigen, 2) are capable of exhibiting high affinity binding to the antigen, 3) possess long half-lives *in vivo*, and, since they are natural immune products, should 4) exhibit low *in vivo* toxicity (Park and Smolen. In: Advances in Protein Chemistry. Academic Press. pp:360-421 (2001)). Antibodies derived from non-human sources, e.g.: mouse, may, however, effect a directed immune response against the therapeutic antibody, following repeated application, thereby neutralizing the antibody's effectiveness. Fully human antibodies offer the greatest potential for success as human therapeutics since they would likely be less immunogenic than murine or chimeric antibodies in humans, similar to naturally occurring immuno-responsive antibodies. To this end, there is a need to develop high affinity human anti-IGF-IR monoclonal antibodies for therapeutic use.

SUMMARY OF THE INVENTION

[0012] The invention provides human monoclonal antibodies and fragments thereof that bind specifically to the human IGF-I receptor. The antibodies have at least one property selected from (i) inhibits binding of IGF-I or IGF-II to IGF-IR, (ii) neutralizes activation of IGF-IR by IGF-I or IGF-II, (iii) reduces IGF-IR surface receptor by at least about 80%; and (iv) binds to IGF-IR with a Kd of about 3×10^{-10} or less. In a more preferred embodiment, an antibody of the invention reduces IGF-IR surface receptor by at least about 85%, and more

preferably by at least about 90%. Further, the antibodies inhibit ligand-mediated receptor autophosphorylation and downstream cellular signaling through the MAPK and Akt pathways. Antibodies of the invention, used alone or in combination with an anti-neoplastic agent, are particularly useful for treating neoplastic diseases and hyperproliferative disorders.

[0013] The invention provides isolated polynucleotides encoding the antibodies or fragments thereof, expression vectors comprising the polynucleotide sequences, and host cells for expression.

[0014] Further, the invention provides pharmaceutical compositions and diagnostic and therapeutic methods for treatment of tumors and hyperproliferative disease. The methods can further comprise administration of an anti-neoplastic agent or treatment.

BRIEF DESCRIPTION OF THE FIGURES

[0015] Figure 1 depicts the nucleotide sequence of the 2F8 heavy chain variable domain.

[0016] Figure 2 depicts the amino acid sequence of the 2F8 heavy chain variable domain. CDRs are in bold and underlined.

[0017] Figure 3 depicts the nucleotide sequence of the complete 2F8 heavy chain (underline: secretory signal sequence; italics: IgG1 constant region).

[0018] Figure 4 depicts the amino acid sequence of the complete 2F8 heavy chain (underline: secretory signal sequence; bold: CDRs; italics: IgG1 constant region).

[0019] Figure 5 depicts the nucleotide sequence of the 2F8 light chain variable domain.

[0020] Figure 6 depicts the amino acid sequence of the 2F8 light chain variable domain. CDRs are in bold and underlined.

[0021] Figure 7 depicts the nucleotide sequence of the complete 2F8 light chain (underline: secretory signal sequence; italics: IgG1 constant region).

[0022] Figure 8 depicts the amino acid sequence of the complete 2F8 light chain (underline: secretory signal sequence; bold: CDRs; italics: IgG1 constant region).

[0023] Figure 9 depicts the nucleotide sequence of the A12 light chain variable domain.

[0024] Figure 10 depicts the amino acid sequence of the A12 light chain variable domain. CDRs are in bold and underlined.

[0025] Fig. 11 depicts the nucleotide sequence of the complete A12 light chain (underline: secretory signal sequence; italics: IgG1 constant region).

[0026] Figure 12 depicts the amino acid sequence of the complete A12 light chain (underline: secretory signal sequence; bold: CDRs; italics: IgG1 constant region).

[0027] Figure 13 depicts V_H and V_L CDR sequences of antibodies 2F8 and A12. Differences between the V_L CDRs are underlined.

[0028] Figure 14 depicts the homology between 2F8 and A12 light chain variable region amino acid sequences. Sequences differences are boxed and CDRs are highlighted.

[0029] Figure 15 shows results of an assay that measures the capacity of antibodies 2F8 and A12 to block binding of IGF-I to immobilized soluble IGF-IR. A12 antibodies having kappa or lambda light chain constant regions were tested.

[0030] Figure 16 shows results of an assay that measures the capacity of antibodies 2F8 and A12 (lambda light chain) to block binding of IGF-I to MCF7 cells.

[0031] Figure 17 shows results of an assay that measures the capacity of antibodies 2F8 and A12 to block insulin binding to ZR75-I cells.

[0032] Figure 18 shows the effect of antibody A12 on ^{3}H-thymidine incorporation in a mitogenesis assay. Figure 18A: MCF7 breast cancer cells; Figure 18B: BxPC-3 pancreatic cancer cells; Figure 18C: HT-29 colon cancer cells.

[0033] Figure 19 shows inhibition of IGF-I mediated receptor phosphorylation. Panel A: Inhibition in MCF7 breast cancer cells by antibodies A12 and 2F8. Panel B: Inhibition in HT-29 colorectal cancer cells and BxPC-3 pancreatic cancer cells by antibody A12.

[0034] Figure 20 shows inhibition of IGF-I mediated phosphorylation of downstream effector molecules by antibodies A12 and 2F8. Panel A: inhibition of phosphorylation of MAPK; Panel B: inhibition of phosphorylation of Akt.

[0035] Figure 21 shows binding of antibody A12 to human and murine IGF-IR positive and negative cell lines. MCF7: human breast cancer cells; R-: mouse embryo fibroblasts; HEL: human leukemia cells; Lewis Lung: mouse lung carcinoma cells.

[0036] Figure 22 shows receptor internalization. Panel A shows internalization of labeled antibody A12 following binding to IGF-IR on MCF7 cells. Panel B shows depletion of cell-surface associated IGF-IR. Panel C shows degradation of total cellular IGF-IR after prolonged treatment with A12.

[0037] Figure 23 shows inhibition of HT-29 human colon carcinoma growth in nude mice by antibody 2F8 and CPT-11 (irinotecan) alone or in combination.

[0038] Figure 24 shows the effect of antibody A12 on HT-29 human colorectal tumor growth in nude mice.

[0039] Figure 25 shows the effect of antibody A12 on MCF7 human breast cancer growth in nude mice.

[0040] Figure 26 shows inhibition of BxPC-3 pancreatic cancer xenografts in nude mice by antibody A12, gemcitabine, or CPT-11 (irinotecan) alone, or in combination.

[0041] Figure 27 shows inhibition of HT-29 colorectal cancer xenografts in nude mice by antibody A12, paclitaxel, or CPT-11 (irinotecan) alone, or in combination.

DETAILED DESCRIPTION OF THE INVENTION

[0042] The present invention provides antibodies and fragments thereof, specific for insulin-like growth factor-I receptor (IGF-IR), as well as isolated or purified polynucleotide sequences encoding the antibodies. In certain embodiments of the invention, human antibodies are provided. The antibodies can also be used in combination with other chemical and biological agents, including, but not limited to, anti-neoplastic agents and/or agents that are inhibitors of other receptors or receptor substrates mediating cell growth. The invention further relates to anti-neoplastic agents that are inhibitors of topoisomerase function. The choice of such agents is advantageous for use in therapeutic methods in combination with antibodies that are specific for IGF-IR.

[0043] Naturally occurring antibodies typically have two identical heavy chains and two identical light chains, with each light chain covalently linked to a heavy chain by an interchain disulfide bond and multiple disulfide bonds further link the two heavy chains to one another. Individual chains can fold into domains having similar sizes (110-125 amino acids) and structures, but different functions. The light chain can comprise one variable domain (V_L) and/or one constant domain (C_L). The heavy chain can also comprise one variable domain (V_H) and/or, depending on the class or isotype of antibody, three or four constant domains (C_H1, C_H2, C_H3 and C_H4). In humans, the isotypes are IgA, IgD, IgE, IgG, and IgM, with IgA and IgG further subdivided into subclasses or subtypes (IgA_{1-2} and IgG_{1-4}).

[0044] Generally, the variable domains show considerable amino acid sequence variability from one antibody to the next, particularly at the location of the antigen-binding site. Three regions, called hypervariable or complementarity-determining regions (CDRs), are found in each of V_L and V_H, which are supported by less variable regions called frameworks.

[0045] The portion of an antibody consisting of V_L and V_H domains is designated Fv (Fragment variable) and constitutes the antigen-binding site. Single chain Fv (scFv) is an antibody fragment containing a V_L domain and a V_H domain on one polypeptide chain, wherein the N terminus of one domain and the C terminus of the other domain are joined by a flexible linker (see, e.g., U.S. Pat. No. 4,946,778 (Ladner et al.); WO 88/09344, (Huston et al.). WO 92/01047 (McCafferty et al.) describes the display of scFv fragments on the surface of soluble recombinant genetic display packages, such as bacteriophage.

[0046] The peptide linkers used to produce the single chain antibodies can be flexible peptides selected to assure that the proper three-dimensional folding and association of the V_L and V_H domains occurs. The linker is generally 10 to 50 amino acid residues. Preferably, the linker is 10 to 30 amino acid residues. More preferably the linker is 12 to 30 amino acid residues. Most preferably is a linker of 15 to 25 amino acid residues. An non-limiting example of such a linker peptides is $(Gly\text{-}Gly\text{-}Gly\text{-}Gly\text{-}Ser)_3$ (SEQ ID NO:33).

[0047] Fab (Fragment, antigen binding) refers to the fragments of the antibody consisting of V_L-C_L and V_H-C_H1 domains. Such a fragment generated by digestion of a whole antibody with papain does not retain the antibody hinge region by which two heavy chains are normally linked. The fragment is monovalent and simply referred to as Fab. Alternatively, digestion with pepsin results in a fragment that retains the hinge region. Such a fragment with intact interchain disulfide bonds linking two heavy chains is divalent and is referred to as $F(ab')_2$. A monovalent Fab' results when the disulfide bonds of an $F(ab')_2$ are reduced (and the heavy chains are separated. Because they are divalent, intact antibodies and $F(ab')_2$ fragments have higher avidity for antigen that the monovalent Fab or Fab' fragments. WO 92/01047 (McCafferty et al.) describes the display of Fab fragments on the surface of soluble recombinant genetic display packages, such as bacteriophage.

[0048] Fc (Fragment crystallization) is the designation for the portion or fragment of an antibody that consists of paired heavy chain constant domains. In an IgG antibody, for example, the Fc consists of heavy chain C_H2 and C_H3 domains. The Fc of an IgA or an IgM

antibody further comprises a C_H4 domain. The Fc is associated with Fc receptor binding, activation of complement-mediated cytotoxicity and antibody-dependent cellular-cytotoxicity (ADCC). For antibodies such as IgA and IgM, which are complexes of multiple IgG like proteins, complex formation requires Fc constant domains.

[0049] Finally, the hinge region separates the Fab and Fc portions of the antibody, providing for mobility of Fabs relative to each other and relative to Fc, as well as including multiple disulfide bonds for covalent linkage of the two heavy chains.

[0050] Antibody formats have been developed which retain binding specificity, but have other characteristics that may be desirable, including for example, bispecificity, multivalence (more than two binding sites), compact size (e.g., binding domains alone).

[0051] Single chain antibodies lack some or all of the constant domains of the whole antibodies from which they are derived. Therefore, they can overcome some of the problems associated with the use of whole antibodies. For example, single-chain antibodies tend to be free of certain undesired interactions between heavy-chain constant regions and other biological molecules. Additionally, single-chain antibodies are considerably smaller than whole antibodies and can have greater permeability than whole antibodies, allowing single-chain antibodies to localize and bind to target antigen-binding sites more efficiently. Furthermore, the relatively small size of single-chain antibodies makes them less likely to provoke an unwanted immune response in a recipient than whole antibodies.

[0052] Multiple single chain antibodies, each single chain having one V_H and one V_L domain covalently linked by a first peptide linker, can be covalently linked by at least one or more peptide linker to form a multivalent single chain antibodies, which can be monospecific or multispecific. Each chain of a multivalent single chain antibody includes a variable light chain fragment and a variable heavy chain fragment, and is linked by a peptide linker to at least one other chain. The peptide linker is composed of at least fifteen amino acid residues. The maximum number of amino acid residues is about one hundred.

[0053] Two single chain antibodies can be combined to form a diabody, also known as a bivalent dimer. Diabodies have two chains and two binding sites, and can be monospecific or bispecific. Each chain of the diabody includes a V_H domain connected to a V_L domain. The domains are connected with linkers that are short enough to prevent pairing between domains on the same chain, thus driving the pairing between complementary domains on different chains to recreate the two antigen-binding sites.

[0054] Three single chain antibodies can be combined to form triabodies, also known as trivalent trimers. Triabodies are constructed with the amino acid terminus of a V_L or V_H domain directly fused to the carboxyl terminus of a V_L or V_H domain, i.e., without any linker sequence. The triabody has three Fv heads with the polypeptides arranged in a cyclic, head-to-tail fashion. A possible conformation of the triabody is planar with the three binding sites located in a plane at an angle of 120 degrees from one another. Triabodies can be monospecific, bispecific or trispecific.

[0055] Thus, antibodies of the invention and fragments thereof include, but are not limited to, naturally occurring antibodies, bivalent fragments such as $(Fab')_2$, monovalent fragments such as Fab, single chain antibodies, single chain Fv (scFv), single domain antibodies, multivalent single chain antibodies, diabodies, triabodies, and the like that bind specifically with antigens.

[0056] The antibodies of the present invention and particularly the variable domains thereof may be obtained by methods known in the art. These methods include, for example, the immunological method described by Kohler and Milstein, Nature, 256: 495-497 (1975) and Campbell, Monoclonal Antibody Technology, The Production and Characterization of Rodent and Human Hybridomas, Burdon et al., Eds., Laboratory Techniques in Biochemistry and Molecular Biology, Volume 13, Elsevier Science Publishers, Amsterdam (1985); as well as by the recombinant DNA methods such as described by Huse et al., Science, 246, 1275-81 (1989). The antibodies can also be obtained from phage display libraries bearing combinations of V_H and V_L domains in the form of scFv or Fab. The V_H and V_L domains can be encoded by nucleotides that are synthetic, partially synthetic, or naturally derived. In certain embodiments, phage display libraries bearing human antibody fragments can be preferred. Other sources of human antibodies are transgenic mice engineered to express human immunoglobulin genes.

[0057] Antibody fragments can be produced by cleaving a whole antibody, or by expressing DNA that encodes the fragment. Fragments of antibodies may be prepared by methods described by Lamoyi et al., *J. Immunol. Methods*, 56: 235-243 (1983) and by Parham, *J. Immunol.* 131: 2895-2902 (1983). Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Such fragments may also contain single-chain fragment variable region antibodies, i.e. scFv, dibodies, or other antibody fragments. Methods of producing such functional equivalents are disclosed in PCT Application WO 93/21319,

European Patent Application No. 239,400; PCT Application WO 89/09622; European Patent Application 338,745; and European Patent Application EP 332,424.

[0058] The antibodies, or fragments thereof, of the present invention are specific for IGF-IR. Antibody specificity refers to selective recognition of the antibody for a particular epitope of an antigen. Antibodies, or fragments thereof, of the present invention, for example, can be monospecific or bispecific. Bispecific antibodies (BsAbs) are antibodies that have two different antigen-binding specificities or sites. Where an antibody has more than one specificity, the recognized epitopes can be associated with a single antigen or with more than one antigen. Thus, the present invention provides bispecific antibodies, or fragments thereof, that bind to two different antigens, with at least one specificity for IGF-IR.

[0059] Specificity of the present antibodies, or fragments thereof, for IGF-IR can be determined based on affinity and/or avidity. Affinity, represented by the equilibrium constant for the dissociation of an antigen with an antibody (Kd), measures the binding strength between an antigenic determinant and an antibody-binding site. Avidity is the measure of the strength of binding between an antibody with its antigen. Avidity is related to both the affinity between an epitope with its antigen binding site on the antibody, and the valence of the antibody, which refers to the number of antigen binding sites specific for a particular epitope. Antibodies typically bind with a dissociation constant (Kd) of 10^{-5} to 10^{-11} liters/mol. Any Kd greater than 10^{-4} liters/mol is generally considered to indicate nonspecific binding. The lesser the value of the Kd, the stronger the binding strength between an antigenic determinant and the antibody binding site.

[0060] Antibodies of the present invention, or fragments thereof, also include those for which binding characteristics have been improved by direct mutation, methods of affinity maturation, phage display, or chain shuffling. Affinity and specificity can be modified or improved by mutating CDR and/or FW residues and screening for antigen binding sites having the desired characteristics (see, e.g., Yang et al., J. Mol. Biol., (1995) 254: 392-403). One way is to randomize individual residues or combinations of residues so that in a population of, otherwise identical antigen binding sites, subsets of from two to twenty amino acids are found at particular positions. Alternatively, mutations can be induced over a range of residues by error prone PCR methods (see, e.g., Hawkins et al., J. Mol. Biol., (1992) 226: 889-96). In another example, phage display vectors containing heavy and light chain variable region genes can be propagated in mutator strains of E. coli (see, e.g., Low et al., J. Mol.

Biol., (1996) 250: 359-68). These methods of mutagenesis are illustrative of the many methods known to one of skill in the art.

[0061] Equivalents of the antibodies, or fragments thereof, of the present invention include polypeptides with amino acid sequences substantially the same as the amino acid sequence of the variable or hypervariable regions of the full-length anti-IGF-IR antibodies. Substantially the same amino acid sequence is defined herein as a sequence with at least 70%, preferably at least about 80%, and more preferably at least about 90% homology to another amino acid sequence, as determined by the FASTA search method in accordance with Pearson and Lipman (Proc. Natl. Acad. Sci. USA (1988) 85: 2444-8).

[0062] Conservative amino acid substitution is defined as a change in the amino acid composition by way of changing one or two amino acids of a peptide, polypeptide or protein, or fragment thereof. The substitution is of amino acids with generally similar properties (e.g., acidic, basic, aromatic, size, positively or negatively charged, polarity, non-polarity) such that the substitutions do not substantially alter peptide, polypeptide or protein characteristics (e.g., charge, isoelectric point, affinity, avidity, conformation, solubility) or activity. Typical substitutions that may be performed for such conservative amino acid substitution may be among the groups of amino acids as follows:

glycine (G), alanine (A), valine (V), leucine (L) and isoleucine (I);

aspartic acid (D) and glutamic acid (E);

alanine (A), serine (S) and threonine (T);

histidine (H), lysine (K) and arginine (R):

asparagine (N) and glutamine (Q);

phenylalanine (F), tyrosine (Y) and tryptophan (W)

[0063] Conservative amino acid substitutions can be made in, e.g., regions flanking the hypervariable regions primarily responsible for the selective and/or specific binding characteristics of the molecule, as well as other parts of the molecule, e.g., variable heavy chain cassette.

[0064] Each domain of the antibodies of this invention can be a complete antibody with the heavy or light chain variable domain, or it can be a functional equivalent or a mutant or derivative of a naturally-occurring domain, or a synthetic domain constructed, for example, *in vitro* using a technique such as one described in WO 93/11236 (Griffiths et al.). For instance, it is possible to join together domains corresponding to antibody variable

domains, which are missing at least one amino acid. The important characterizing feature is the ability of each domain to associate with a complementary domain to form an antigen-binding site. Accordingly, the terms variable heavy and light chain fragment should not be construed to exclude variants that do not have a material effect on specificity.

[0065] In a preferred embodiment, the anti-IGF-IR antibodies of the present invention are human antibodies that exhibit one or more of following properties.

[0066] 1) The antibodies bind to the external domain of IGF-IR and inhibit binding of IGF-I or IGF-II to IGF-IR. Inhibition can be determined, for example, by a direct binding assay using purified or membrane bound receptor. In this embodiment, the antibodies of the present invention, or fragments thereof, preferably bind IGF-IR at least as strongly as the natural ligands of IGF-IR (IGF-I and IGF-II).

[0067] 2) The antibodies neutralize IGF-IR. Binding of a ligand, *e.g.*, IGF-I or IGF-II, to an external, extracellular domain of IGF-IR stimulates autophosphorylation of the beta subunit and phosphorylation of IFG-IR substrates, including MAPK, Akt, and IRS-1.

[0068] Neutralization of IGF-IR includes inhibition, diminution, inactivation and/or disruption of one or more of these activities normally associated with signal transduction. Further, this includes inhibition of IGF-IR / IR heterodimers as well as IGF-IR homodimers. Thus, neutralizing IGF-IR has various effects, including inhibition, diminution, inactivation and/or disruption of growth (proliferation and differentiation), angiogenesis (blood vessel recruitment, invasion, and metastasis), and cell motility and metastasis (cell adhesion and invasiveness).

[0069] One measure of IGF-IR neutralization is inhibition of the tyrosine kinase activity of the receptor. Tyrosine kinase inhibition can be determined using well-known methods; for example, by measuring the autophosphorylation level of recombinant kinase receptor, and/or phosphorylation of natural or synthetic substrates. Thus, phosphorylation assays are useful in determining neutralizing antibodies in the context of the present invention. Phosphorylation can be detected, for example, using an antibody specific for phosphotyrosine in an ELISA assay or on a western blot. Some assays for tyrosine kinase activity are described in Panek et al., *J. Pharmacol. Exp. Thera.* 283: 1433-44 (1997) and Batley et al., *Life Sci.* 62:143-50 (1998). Antibodies of the invention cause a decrease in tyrosine phosphorylation of IGF-IR of at least about 75%, preferably at least about 85%, and more preferably at least about 90% in cells that respond to ligand.

[0070] Another measure of IGF-IR neutralization is inhibition of phosphorylation of downstream substrates of IGF-IR. Accordingly, the level of phosphorylation of MAPK, Akt, or IRS-1 can be measured. The decrease in substrate phosphorylation is at least about 50%, preferably at least about 65%, more preferably at least about 80%.

[0071] In addition, methods for detection of protein expression can be utilized to determine IGF-IR neutralization, wherein the proteins being measured are regulated by IGF-IR tyrosine kinase activity. These methods include immunohistochemistry (IHC) for detection of protein expression, fluorescence in situ hybridization (FISH) for detection of gene amplification, competitive radioligand binding assays, solid matrix blotting techniques, such as Northern and Southern blots, reverse transcriptase polymerase chain reaction (RT-PCR) and ELISA. See, e.g., Grandis et al., *Cancer*, 78:1284-92 (1996); Shimizu et al., *Japan J. Cancer Res.*, 85:567-71 (1994); Sauter et al., *Am. J. Path.*, 148:1047-53 (1996); Collins, *Glia* 15:289-96 (1995); Radinsky et al., *Clin. Cancer Res.* 1:19-31 (1995); Petrides et al., *Cancer Res.* 50:3934-39 (1990); Hoffmann et al., *Anticancer Res.* 17:4419-26 (1997); Wikstrand et al., *Cancer Res.* 55:3140-48 (1995).

[0072] *In vivo* assays can also be utilized to determine IGF-IR neutralization. For example, receptor tyrosine kinase inhibition can be observed by mitogenic assays using cell lines stimulated with receptor ligand in the presence and absence of inhibitor. For example, MCF7 (American Type Culture Collection (ATCC), Rockville, MD) stimulated with IGF-I or IGF-II can be used to assay IGF-IR inhibition. Another method involves testing for inhibition of growth of IGF-IR -expressing tumor cells or cells transfected to express IGF-IR. Inhibition can also be observed using tumor models, for example, human tumor cells injected into a mouse.

[0073] The present invention is not limited by any particular mechanism of IGF-IR neutralization. The anti-IGF-IR antibodies of the present invention can bind externally to the IGF-IR cell surface receptor, block binding of ligand (e.g., IGF-I or IGF-II) and subsequent signal transduction mediated via the receptor-associated tyrosine kinase, and prevent phosphorylation of the IGF-IR and other downstream proteins in the signal transduction cascade.

[0074] 3) The antibodies down modulate IGF-IR. The amount of IGF-IR present on the surface of a cell depends on receptor protein production, internalization, and degradation. The amount of IGF-IR present on the surface of a cell can be measured indirectly, by

detecting internalization of the receptor or a molecule bound to the receptor. For example, receptor internalization can be measured by contacting cells that express IGF-IR with a labeled antibody. Membrane-bound antibody is then stripped, collected and counted. Internalized antibody is determined by lysing the cells and detecting label in the lysates.,

[0075] Another way is to directly measure the amount of the receptor present on the cell following treatment with an anti-IGF-IR antibody or other substance, for example, by fluorescence-activated cell-sorting analysis of cells stained for surface expression of IGF-IR. Stained cells are incubated at 37°C and fluorescence intensity measured over time. As a control, part of the stained population can be incubated at 4°C (conditions under which receptor internalization is halted).

[0076] As described in the Examples, cell surface IGF-IR can be detected and measured using a different antibody that is specific for IGF-IR and that does not block or compete with binding of the antibody being tested. (Burtrum, et al. *Cancer Res.* 63:8912-21 (2003)) Treatment of an IGF-IR expressing cell with an antibody of the invention results in reduction of cell surface IGF-IR. In a preferred embodiment, the reduction is at least about 70%, more preferably at least about 80%, and even more preferably at least about 90% in response to treatment with an antibody of the invention. A significant decrease can be observed in as little as four hours.

[0077] Another measure of down-modulation is reduction of the total receptor protein present in a cell, and reflects degradation of internal receptors. Accordingly, treatment of cells (particularly cancer cells) with antibodies of the invention results in a reduction in total cellular IGF-IR. In a preferred embodiment, the reduction is at least about 70%, more preferably at least about 80%, and even more preferably at least about 90%.

[0078] The antibodies of the invention bind to IGF-IR with a K_d of about 3×10^{-10} M^{-1} or less, preferably about 1×10^{-10} M^{-1} or less, and more preferably about 3×10^{-11} M^{-1} or less.

[0079] In an embodiment of the invention, the antibodies inhibit tumor growth. For example, subcutaneous xenograft tumors can be established by injection of cells of a cancer cell line into an immunodeficient mouse. The mice are then treated by intraperitoneal injection of antibodies, for example, every three days, and tumor size measured at regular intervals. Compared to control injections, antibodies of the invention inhibit tumor growth. In a preferred embodiment, an antibody of the invention promotes tumor regression when

combined with an anti-neoplastic agent. Further, as exemplified below, in a more preferred embodiment, antibodies of the invention promoting tumor regression when used in a monotherapy. By promoting tumor regression is meant that administration of an effective amount of antibody, or an effective amount of a combination of an antibody and a neoplastic agent results in a reduction is size or necrosis of the tumor. In a preferred embodiment of the invention, tumor regression may be observed and continue for a period of at least about 20 days, more preferably at least about 40 days, more preferably at least about 60 days. Tumor regression may can be measured as an average across a group of subjects undergoing a particular treatment regimen, or can be measured by the number of subjects in a treatment group in which tumors regress.

[0080] Preferred antibodies of the present invention, or fragments thereof, are human antibodies having one, two, three, four, five, and/or six complementarity determining regions (CDRs) selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28,and SEQ ID NO:30. Preferably, the antibodies (or fragments thereof) of the present invention have CDRs of SEQ ID NO:14, SEQ ID NO:16 and SEQ ID NO:18. Alternatively and also preferably, the present antibodies, or fragments thereof, have CDRs of SEQ ID NO:20, SEQ ID NO:22 and SEQ ID NO:24. Alternatively and also preferably, the present antibodies, or fragments thereof, have CDRs of SEQ ID NO:26, SEQ ID NO:28 and SEQ ID NO:30. The amino acid sequences of the CDRs are set forth below in Table 1.

TABLE 1

Heavy Chain (2F8/A12)		
CDR1	SYAIS	SEQ ID NO:14
CDR2	GIIPIFGTANYAQKFQG	SEQ ID NO:16
CDR3	APLRFLEWSTQDHYYYYYMDV	SEQ ID NO:18
Light Chain (2F8)		
CDR1	QGDSLRSYYAS	SEQ ID NO:20
CDR2	GKNNRPS	SEQ ID NO:22
CDR3	NSRDNSDNRLI	SEQ ID NO:24
Light Chain (A12)		
CDR1	QGDSLRSYYAT	SEQ ID NO:26
CDR2	GENKRPS	SEQ ID NO:28
CDR3	KSRDGSGQHLV	SEQ ID NO:30

[0081] In another embodiment, the present antibodies, or fragments thereof, can have a heavy chain variable region of SEQ ID NO:1 and/or a light chain variable region selected from SEQ ID NO:5 or SEQ ID NO:6. IMC-A12 is a particularly preferred antibody of the present invention. This antibody has human V_H and V_L framework regions (FWs) as well as CDRs. The V_H variable domain of IMC-A12 (SEQ ID NO:1) has three CDRs corresponding to SEQ ID NOs:14, 16, and 18 and the V_L domain (SEQ ID NO:5) has three CDRs corresponding to SEQ ID NOS:20, 22, and 24. IMC-2F8 is another preferred antibody of the present invention. This antibody also has human V_H and V_L framework regions (FWs) and CDRs. The V_H variable domain of IMC-2F8 is identical to the V_H variable domain of IMC-A12. The V_L domain of IMC-2F8 (SEQ ID NO:9) has three CDRs corresponding to SEQ ID NOS:26, 28, and 30.

[0082] In another embodiment, antibodies of the invention compete for binding to IGF-IR with IMC-A12 and/or IMC-2F8. That is, the antibodies bind to the same or similar overlapping epitope.

[0083] The present invention also provides isolated polynucleotides encoding the antibodies, or fragments thereof, described previously. The invention includes nucleic acids having a sequence encoding one, two, three, four, five and/or all six CDRs as set forth in Table 2.

TABLE 2

Heavy Chain (2F8/A12)		
CDR1	agctatgcta tcagc	SEQ ID NO:13
CDR2	gggatcatcc ctatctttgg tacagcaaac tacgcacaga agttccaggg c	SEQ ID NO:15
CDR3	gcgccattac gatttttgga gtggtccacc caagaccact actactacta ctacatg gacgtc	SEQ ID NO:17
Light Chain (2F8)		
CDR1	caaggagaca gcctcagaag ctattatgca agc	SEQ ID NO:19
CDR2	ggtaaaaaca accggccctc a	SEQ ID NO:21
CDR3	aactcccggg acaacagtga taaccgtctg ata	SEQ ID NO:23
Light Chain (A12)		
CDR1	caaggagaca gcctcagaag ctattatgca acc	SEQ ID NO:25
CDR2	ggtgaaaata agcggccctc a	SEQ ID NO:27
CDR3	aaatctcggg atggcagtgg tcaacatctg gtg	SEQ ID NO:29

[0084] DNA encoding human antibodies can be prepared by recombining DNA encoding human constant regions and variable regions, other than the CDRs, derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived from a human (e.g., SEQ ID NOs:13, 15, and 17 for the heavy chain variable domain CDRs and SEQ ID NOs:19, 21, and 23 or SEQ ID NOS:25, 27 and 29 for the light chain variable domain CDRs).

[0085] Other suitable sources of DNAs that encode fragments of antibodies include any cell, such as hybridomas and spleen cells, that express the full-length antibody. The fragments may be used by themselves as antibody equivalents, or may be recombined into equivalents, as described above. The DNA recombinations and other techniques described in this section may be carried out by known methods. Other sources of DNAs are single chain antibodies or Fabs produced from a phage display library, as is known in the art.

[0086] Additionally, the present invention provides expression vectors containing the polynucleotide sequences previously described operably linked to an expression sequence, a promoter and an enhancer sequence. A variety of expression vectors for the efficient synthesis of antibody polypeptide in prokaryotic, such as bacteria and eukaryotic systems, including but not limited to yeast and mammalian cell culture systems have been developed.

The vectors of the present invention can comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences.

[0087] Any suitable expression vector can be used. For example, prokaryotic cloning vectors include plasmids from *E. coli*, such as *colE1*, *pCR1*, *pBR322*, *pMB9*, *pUC*, *pKSM*, and *RP4*. Prokaryotic vectors also include derivatives of phage DNA such as *M13* and other filamentous single-stranded DNA phages. An example of a vector useful in yeast is the 2μ plasmid. Suitable vectors for expression in mammalian cells include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and shuttle vectors derived from combination of functional mammalian vectors, such as those described above, and functional plasmids and phage DNA.

[0088] Additional eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, *J. Mol. Appl. Genet.* 1: 327-41 (1982); Subramani et al., *Mol. Cell. Biol.* 1: 854-64 (1981); Kaufmann and Sharp, "Amplification And Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene," *J. Mol. Biol.* 159: 601-21 (1982); Kaufmann and Sharp, *Mol. Cell. Biol.* 159: 601-64 (1982); Scahill et al., "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," *Proc. Nat'l Acad. Sci. USA* 80, 4654-59 (1983); Urlaub and Chasin, *Proc. Nat'l Acad. Sci. USA* 77: 4216-20, (1980).

[0089] The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the *lac* system, the *trp* system, the *tac* system, the *trc* system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

[0090] Where it is desired to express a gene construct in yeast, a suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7. Stinchcomb et al. *Nature*, 282: 39 (1979); Kingsman et al., *Gene*, 7: 141 (1979). The trp1 gene provides a

selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics*, 85: 12 (1977). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

[0091] The present invention also provides recombinant host cells containing the expression vectors previously described. Antibodies of the present invention can be expressed in cell lines other than in hybridomas. Nucleic acids, which comprise a sequence encoding a polypeptide according to the invention, can be used for transformation of a suitable mammalian host cell.

[0092] Cell lines of particular preference are selected based on high level of expression, constitutive expression of protein of interest and minimal contamination from host proteins. Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines, such as but not limited to, COS-7 cells, Chinese Hamster Ovary (CHO) cells, Baby Hamster Kidney (BHK) cells and many others including cell lines of lymphoid origin such as lymphoma, myeloma, or hybridoma cells. Suitable additional eukaryotic cells include yeast and other fungi. Useful prokaryotic hosts include, for example, *E. coli*, such as *E. coli* SG-936, *E. coli* HB 101, *E. coli* W3110, *E. coli* X1776, *E. coli* X2282, *E. coli* DHI, and *E. coli* MRCl, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis*, and *Streptomyces*.

[0093] These present recombinant host cells can be used to produce an antibody, or fragment thereof, by culturing the cells under conditions permitting expression of the antibody or fragment thereof and purifying the antibody or fragment thereof from the host cell or medium surrounding the host cell. Targeting of the expressed antibody or fragment for secretion in the recombinant host cells can be facilitated by inserting a signal or secretory leader peptide-encoding sequence (see, Shokri et al., *Appl Microbiol Biotechnol.* 60:654-64 (2003) Nielsen et al., *Prot. Eng.* 10:1-6 (1997) and von Heinje et al., *Nucl. Acids Res.* 14:4683-90 (1986)) at the 5' end of the antibody-encoding gene of interest. These secretory leader peptide elements can be derived from either prokaryotic or eukaryotic sequences. Accordingly suitably, secretory leader peptides are used, being amino acids joined to the N-

terminal end of a polypeptide to direct movement of the polypeptide out of the host cell cytosol and secretion into the medium.

[0094] The transformed host cells are cultured by methods known in the art in a liquid medium containing assimilable sources of carbon (carbohydrates such as glucose or lactose), nitrogen (amino acids, peptides, proteins or their degradation products such as peptones, ammonium salts or the like), and inorganic salts (sulfates, phosphates and/or carbonates of sodium, potassium, magnesium and calcium). The medium furthermore contains, for example, growth-promoting substances, such as trace elements, for example iron, zinc, manganese and the like.

[0095] Another embodiment for the preparation of antibodies in the present invention is the expression of the nucleic acid encoding the antibody according to the invention in a transgenic animal that has a substantial portion of the human antibody producing genome inserted and is rendered deficient in the production of endogenous antibodies. Transgenic animals, include but not limited to mice, goat, and rabbit. One further embodiment of the invention, include expression of the antibody-coding gene in, for example, the mammary gland of the animal for secretion of the polypeptide during lactation.

[0096] As described in the examples below, high affinity anti-IGF-IR antibodies according to the present invention can be isolated from a phage display library constructed from human heavy chain and light chain variable region genes. For example, a variable domain of the invention can be obtained from a peripheral blood lymphocyte that contains a rearranged variable region gene. Alternatively, variable domain portions, such as CDR and FW regions, can be derived from different human sequences. Over 90% of recovered clones after three rounds of selection are specific to IGF-IR. The binding affinities for IGF-IR of the screened Fabs can be in the nM range, which is as high as many bivalent anti- IGF-IR monoclonal antibodies produced using hybridoma technology.

[0097] Antibodies, and fragments thereof, of the present invention can be obtained, for example, from naturally occurring antibodies, or Fab or scFv phage display libraries. Single domain antibodies can be obtained by selecting a V_H or a V_L domain from a naturally occurring antibody or hybridoma, or selected from a library of V_H domains or a library of V_L domains. It is understood that amino acid residues that are primary determinants of binding of single domain antibodies can be within Kabat defined CDRs, but may include other

residues as well, such as, for example, residues that would otherwise be buried in the V_H-V_L interface of a V_H-V_L heterodimer.

[0098] Antibodies of the present invention also include those for which binding characteristics have been improved by direct mutation, methods of affinity maturation, phage display, or chain shuffling. Affinity and specificity may be modified or improved by mutating CDRs and screening for antigen binding sites having the desired characteristics (see, e.g.; Yang et al., J. Mol. Biol., 254: 392-403 (1995)). CDRs are mutated in a variety of ways. One way is to randomize individual residues or combinations of residues so that in a population of otherwise identical antigen binding sites, all twenty amino acids are found at particular positions. Alternatively, mutations are induced over a range of CDR residues by error prone PCR methods (see, e.g., Hawkins et al., J. Mol. Biol., 226: 889-896 (1992)). For example, phage display vectors containing heavy and light chain variable region genes may be propagated in mutator strains of E. coli (see, e.g., Low et al., J. Mol. Biol., 250: 359-368 (1996)). These methods of mutagenesis are illustrative of the many methods known to one of skill in the art.

[0099] The protein used to identify IGF-IR binding antibodies of the invention is preferably IGF-RI and, more preferably, is the extracellular domain of IGF-RI. The IGF-RI extracellular domain can be free or conjugated to another molecule.

[0100] The antibodies of this invention can be fused to additional amino acid residues. Such amino acid residues can be a peptide tag, perhaps to facilitate isolation. Other amino acid residues for homing of the antibodies to specific organs or tissues are also contemplated.

[0101] In another aspect of the invention, anti-IGF-IR antibodies or antibody fragments can be chemically or biosynthetically linked to anti-tumor agents or detectable signal-producing agents. As exemplified below, antibodies of the invention are efficiently internalized upon binding to cells bearing IGF-IR. Anti-tumor agents linked to an antibody include any agents which destroy or damage a tumor to which the antibody has bound or in the environment of the cell to which the antibody has bound. For example, an anti-tumor agent is a toxic agent such as a chemotherapeutic agent or a radioisotope. Suitable chemotherapeutic agents are known to those skilled in the art and include anthracyclines (e.g. daunomycin and doxorubicin), methotrexate, vindesine, neocarzinostatin, cis-platinum, chlorambucil, cytosine arabinoside, 5-fluorouridine, melphalan, ricin and calicheamicin. The

chemotherapeutic agents are conjugated to the antibody using conventional methods (See, e.g., Hermentin and Seiler, *Behring Inst. Mitt.* 82:197-215(1988)).

[0102] Detectable signal-producing agents are useful in vivo and in vitro for diagnostic purposes. The signal producing agent produces a measurable signal which is detectable by external means, usually the measurement of electromagnetic radiation. For the most part, the signal producing agent is an enzyme or chromophore, or emits light by fluorescence, phosphorescence or chemiluminescence. Chromophores include dyes which absorb light in the ultraviolet or visible region, and can be substrates or degradation products of enzyme catalyzed reactions.

[0103] The invention further contemplates anti-IGF-IR antibodies or antibody fragments of the invention to which target or reporter moieties are linked. Target moieties are first members of binding pairs. Anti-tumor agents, for example, are conjugated to second members of such pairs and are thereby directed to the site where the antigen-binding protein is bound. A common example of such a binding pair is avidin and biotin. In a preferred embodiment, biotin is conjugated to an antigen-binding protein of the invention, and thereby provides a target for an anti-tumor agent or other moiety which is conjugated to avidin or streptavidin. Alternatively, biotin or another such moiety is linked to an antigen-binding protein of the invention and used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin.

[0104] Suitable radioisotopes for use as anti-tumor agents are also known to those skilled in the art. For example, ^{131}I or ^{211}At is used. These isotopes are attached to the antibody using conventional techniques (See, e.g., Pedley et al., *Br. J. Cancer* 68, 69-73(1993)). Alternatively, the anti-tumor agent which is attached to the antibody is an enzyme which activates a prodrug. In this way, a prodrug is administered which remains in its inactive form until it reaches the tumor site where it is converted to its cytotoxin form once the antibody complex is administered. In practice, the antibody-enzyme conjugate is administered to the patient and allowed to localize in the region of the tissue to be treated. The prodrug is then administered to the patient so that conversion to the cytotoxic drug occurs in the region of the tissue to be treated. Alternatively, the anti-tumor agent conjugated to the antibody is a cytokine such as interleukin-2 (IL-2), interleukin-4 (IL-4) or tumor necrosis factor alpha (TNF-α). The antibody targets the cytokine to the tumor so that the cytokine mediates damage to or destruction of the tumor without affecting other tissues. The

cytokine is fused to the antibody at the DNA level using conventional recombinant DNA techniques.

[0105] A method of treating tumor growth in a mammal by administering to the mammal an effective amount of an antibody as previously described is also provided by the present invention. The IGF-IR signaling pathway has been extensively demonstrated to be a causative factor in the development of many types of cancer. IGF-I and IGF-II have been shown to be strong mitogens for a wide variety of cancer cell lines, including prostate, breast, colon, myeloma, ovary, pancreas and lung. Further, highly metastatic cancer cells have been shown to express higher levels of IGF-IR and IGF-II than tumor cells less prone to metastasize.

[0106] Suitable tumors to be treated according to the present invention preferably express IGF-IR. While not intended to be bound to any particular mechanism, the diseases and conditions which can be treated or prevented by the present methods include, for example, those in which pathogenic angiogenesis or tumor growth is stimulated through an IGF-IR paracrine and/or autocrine loop. For example, highly metastatic tumors tend to express both IGF-II and IGF-IR.

[0107] In an embodiment of the invention, anti-IGF-IR antibodies can be administered in combination with one or more other anti-neoplastic agents. For examples of combination therapies, see, e.g., U.S. Patent No. 6,217,866 (Schlessinger et al.) (Anti-EGFR antibodies in combination with anti-neoplastic agents); WO 99/60023 (Waksal et al.) (Anti-EGFR antibodies in combination with radiation). Any suitable anti-neoplastic agent can be used, such as a chemotherapeutic agent, radiation or combinations thereof. The anti-neoplastic agent can be an alkylating agent or an anti-metabolite. Examples of alkylating agents include, but are not limited to, cisplatin, cyclophosphamide, melphalan, and dacarbazine. Examples of anti-metabolites include, but not limited to, doxorubicin, daunorubicin, and paclitaxel, gemcitabine, and topoisomerase inhibitors irinotecan (CPT-11), aminocamptothecin, camptothecin, DX-8951f, and topotecan (topoisomerase I) and etoposide (VP-16) and teniposide (VM-26) (topoisomerase II). When the anti-neoplastic agent is radiation, the source of the radiation can be either external (external beam radiation therapy – EBRT) or internal (brachytherapy – BT) to the patient being treated. The dose of anti-neoplastic agent administered depends on numerous factors, including, for example, the type of agent, the type and severity tumor being treated and the route of administration of the

agent. It should be emphasized, however, that the present invention is not limited to any particular dose.

[0108] The anti-neoplastic agents which are presently known in the art or being evaluated can be grouped into a variety of classes including, for example, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, anti survival agents, biological response modifiers, anti-hormones, and anti-angiogenesis agents.

[0109] Among these classes, the data reported herein suggest that topoisomerase inhibitors are particularly effective anti-neoplastic agents when used in combination with antibodies that bind to IGF-IR. Accordingly, embodiments of the invention include methods in which a topoisomerase inhibitor is administered in combination with an antibody that binds to IGF-IR. The inhibitors can be inhibitors of topoisomerase I or topoisomerase II. Topoisomerase I inhibitors include irinotecan (CPT-11), aminocamptothecin, camptothecin, DX-8951f, topotecan. Topoisomerase II inhibitors include etoposide (VP-16), and teniposide (VM-26). Other substances are currently being evaluated with respect to topoisomerase inhibitory activity and effectiveness as anti-neoplastic agents. In a preferred embodiment, the topoisomerase inhibitor is irinotecan (CPT-11). The antibodies used in combination are antibodies of the invention that bind to IGF-IR and have at least one of the following properties: (i) inhibit binding of IGF-I or IGF-II to IGF-IR; (ii) neutralize activation of IGF-IR by IGF-I or IGF-II; (iii) reduce IGF-IR surface receptor; and bind to IGF-IR with a Kd of about 1×10^{-10} M^{-1} or less. In a more preferred embodiment, the antibodies to be used in combination with a topoisomerase inhibitor have the characteristics of the human antibodies set forth above.

[0110] Anti-IGF-IR antibodies of the invention can be administered with antibodies that neutralize other receptors involved in tumor growth or angiogenesis. In an embodiment of the invention, an anti-IGF-IR antibody is used in combination with a receptor antagonist that binds specifically to EGFR. Particularly preferred are antigen-binding proteins that bind to the extracellular domain of EGFR and block binding of one or more of its ligands and/or neutralize ligand-induced activation of EGFR. An EGFR antagonist can be an antibody that binds to EGFR or a ligand of EGFR and inhibits binding of EGFR to its ligand. Ligands for EGFR include, for example, EGF, TGF-α, amphiregulin, heparin-binding EGF (HB-EGF) and betacellulin. EGF and TGF-α are thought to be the main endogenous ligands that result

in EGFR-mediated stimulation, although TGF-α has been shown to be more potent in promoting angiogenesis. It should be appreciated that the EGFR antagonist can bind externally to the extracellular portion of EGFR, which can or can not inhibit binding of the ligand, or internally to the tyrosine kinase domain. Examples of EGFR antagonists that bind EGFR include, without limitation, biological molecules, such as antibodies (and functional equivalents thereof) specific for EGFR, and small molecules, such as synthetic kinase inhibitors that act directly on the cytoplasmic domain of EGFR.

[0111] Another example of such a receptor is VEGFR. In an embodiment of the present invention, an anti-IGF-IR antibody is used in combination with a VEGFR antagonist. In one embodiment of the invention, an anti-IGF-IR antibody is used in combination with a receptor antagonist that binds specifically to VEGFR-1/Flt-1 receptor. In another embodiment, an anti-IGF-IR antibody is used in combination with a receptor antagonist that binds specifically to VEGFR-2/KDR receptor. Particularly preferred are antigen-binding proteins that bind to the extracellular domain of VEGFR-1 or VEGFR-2 and block binding by their ligands (VEGFR-2 is stimulated most strongly byVEGF; VEGFR-1 is stimulated most strongly by PlGF, but also by VEGF) and/or neutralize ligand-induced induced activation. For example, IMC-1121 is a human antibody that binds to and neutralizes VEGFR-2 (WO 03/075840; Zhu). Another example is MAb 6.12 is a scFv that binds to soluble and cell surface-expressed VEGFR-1. ScFv 6.12 comprises the V_L and V_H domains of mouse monoclonal antibody MAb 6.12. A hybridoma cell line producing MAb 6.12 has been deposited as ATCC number PTA-3344 under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and the regulations thereunder (Budapest Treaty).

[0112] Other examples of growth factor receptors involved in tumorigenesis are the receptors for platelet-derived growth factor (PDGFR), nerve growth factor (NGFR), and fibroblast growth factor (FGFR).

[0113] In an additional alternative embodiment, the IGF-IR antibody can be administered in combination with one or more suitable adjuvants, such as, for example, cytokines (IL-10 and IL-13, for example) or other immune stimulators, such as, but not limited to, chemokine, tumor-associated antigens, and peptides. See, e.g., Larrivée et al., supra. It should be appreciated, however, that administration of only an anti- IGF-IR

antibody is sufficient to prevent, inhibit, or reduce the progression of the tumor in a therapeutically effective manner.

[0114] In a combination therapy, the anti-IGF-IR antibody is administered before, during, or after commencing therapy with another agent, as well as any combination thereof, i.e., before and during, before and after, during and after, or before, during and after commencing the anti-neoplastic agent therapy. For example, the anti-IGF-IR antibody can be administered between 1 and 30 days, preferably 3 and 20 days, more preferably between 5 and 12 days before commencing radiation therapy. In a preferred embodiment of the invention, chemotherapy is administered concurrently with or, more preferably, subsequent to antibody therapy.

[0115] In the present invention, any suitable method or route can be used to administer anti-IGF-IR antibodies of the invention, and optionally, to co-administer anti-neoplastic agents and/or antagonists of other receptors. The anti-neoplastic agent regimens utilized according to the invention, include any regimen believed to be optimally suitable for the treatment of the patient's neoplastic condition. Different malignancies can require use of specific anti-tumor antibodies and specific anti-neoplastic agents, which will be determined on a patient to patient basis. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. The dose of antagonist administered depends on numerous factors, including, for example, the type of antagonists, the type and severity tumor being treated and the route of administration of the antagonists. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

[0116] It is noted that an anti-IGF-IR antibody of the invention can be administered as a conjugate, which binds specifically to the receptor and delivers a toxic, lethal payload following ligand-toxin internalization. The antibody-drug/small molecule conjugate can be directly linked to each other or via a linker, peptide or non-peptide.

[0117] In another aspect of the invention, an anti-IGF-IR antibody of the invention can be chemically or biosynthetically linked to one or more anti-neoplastic or anti-angiogenic agents.

[0118] The invention further contemplates anti-IGF-IR antibodies to which target or reporter moieties are linked. Target moieties are first members of binding pairs. Anti-neoplastic agents, for example, are conjugated to second members of such pairs and are

thereby directed to the site where the anti-IGF-IR antibody is bound. A common example of such a binding pair is avidin and biotin. In a preferred embodiment, biotin is conjugated to an anti-IGF-IR antibody, and thereby provides a target for an anti-neoplastic agent or other moiety, which is conjugated to avidin or streptavidin. Alternatively, biotin or another such moiety is linked to an anti-IGF-IR antibody of the invention and used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin.

[0119] It is understood that the anti-IGF-IR antibodies of the invention, where used in a mammal for the purpose of prophylaxis or treatment, will be administered in the form of a composition additionally comprising a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. Pharmaceutically acceptable carriers can further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the binding proteins. The compositions of the injection can, as is well known in the art, be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the mammal.

[0120] The present invention also includes kits for inhibiting tumor growth and/or angiogenesis comprising a therapeutically effective amount of a human anti-IGF-IR antibody. The kits can further contain any suitable antagonist of, for example, another growth factor receptor involved in tumorigenesis or angiogenesis (e.g., EGFR, VEGFR-1/Flt-1, VEGFR-2, PDGFR, NGFR, FGFR, etc, as described above). Alternatively, or in addition, the kits of the present invention can further comprise an anti-neoplastic agent. Examples of suitable anti-neoplastic agents in the context of the present invention have been described herein. The kits of the present invention can further comprise an adjuvant; examples have also been described above.

[0121] Moreover, included within the scope of the present invention is use of the present antibodies *in vivo* and *in vitro* for investigative or diagnostic methods, which are well known in the art. The diagnostic methods include kits, which contain antibodies of the present invention.

[0122] Accordingly, the present receptor antibodies thus can be used *in vivo* and *in vitro* for investigative, diagnostic, prophylactic, or treatment methods, which are well known

in the art. Of course, it is to be understood and expected that variations in the principles of invention herein disclosed can be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

EXAMPLES

[0123] The following examples further illustrate the invention, but should not be construed to limit the scope of the invention in any way. Detailed descriptions of conventional methods, such as those employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, the introduction of plasmids into host cells, and the expression and determination thereof of genes and gene products can be obtained from numerous publications, including Sambrook, J et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press; and Coligan, J. et al. (1994) Current Protocols in Immunology, Wiley & Sons, Incorporated All references mentioned herein are incorporated in their entirety.

Selection and engineering of anti-human IGF-IR monoclonal antibodies.

[0124] In order to isolate high affinity antibodies to the human IGF-I receptor, recombinant extracellular portion of human IGF-IR was used to screen a human naïve (non-immunized) bacteriophage Fab library containing $3.7x10^{10}$ unique clones (de Haard et al., *J. Biol. Chem.* 274:18218-30 (1999)). Soluble IGF-IR (50µg/ml) was coated onto tubes and blocked with 3% milk/PBS at 37 degrees for 1 hour. Phage were prepared by growing library stock to log phase culture, rescuing with M13K07 helper phage, and amplifying overnight at 30°C in 2YTAK culture medium at containing ampicillin and kanamycin selection. The resulting phage preparation was precipitated in 4% PEG/0.5M NaCl and resuspended in 3% milk/PBS. The immobilized receptors were then incubated with phage preparation for 1 hour at room temperature. Afterwards, the tubes were washed 10 times with PBST (PBS containing 0.1% Tween-20) followed by 10 times with PBS. The bound phage were eluted at RT for 10 min with 1 ml of a freshly prepared solution of 100 mM triethylamine. The eluted phage were incubated with 10 ml of mid-log phase TG1 cells at 37°C for 30 min stationary and 30 min shaking. The infected TG1 cells were pelleted and plated onto several large 2YTAG plates and incubated overnight at 30°C. All colonies that grew on the plates were scraped into 3 to 5 ml of 2YTA medium, mixed with glycerol (final concentration: 10%), aliquoted and stored at -70°C. For second round selection, 100 µl of the phage stock was

added to 25 ml of 2YTAG medium and grown to mid-log phase. The culture was rescued with M13K07 helper phage, amplified, precipitated, and used for selection following the procedure described above, but with reduced concentration (5μg/ml) of IGF-IR immobilized onto tubes and increasing the numbers of washes following the binding process. A total of two rounds of selection were performed.

[0125] Individual TG1 clones were picked and grown at 37°C in 96 well plates and rescued with M13K07 helper phage as described above. The amplified phage preparation was blocked with 1/6 volume of 18% milk/PBS at RT for 1 h and added to Maxi-sorb 96-well microtiter plates (Nunc) coated with IGF-IR (1 μg/ml x 100 μl). After incubation at RT for 1 h the plates were washed 3 times with PBST and incubated with a mouse anti-M13 phage-HRP conjugate (Amersham Pharmacia Biotech, Piscataway, NJ). The plates were washed 5 times, TMB peroxidase substrate (KPL, Gaithersburg, MD) added, and the absorbance at 450 nm read using a microplate reader (Molecular Device, Sunnyvale, CA). From 2 rounds of selection, 80% of independent clones were positive for binding to IGF-IR.

[0126] The diversity of the anti-IGF-IR Fab clones after the second round of selection was analyzed by restriction enzyme digestion pattern (i.e., DNA fingerprint). The Fab gene insert of individual clones was PCR amplified using primers: PUC19 reverse (5'-AGCGGATAACAATTTCACACAGG-3'; SEQ ID NO:31) and fdtet seq (5'-GTCGTCTTTCCAGACGTTAGT-3'; SEQ ID NO:32) which are specific for sequences flanking the unique Fab gene regions within the phage vector. Each amplified product was digested with a frequent-cutting enzyme, *Bst*N I, and analyzed on a 3% agarose gel. A total of 25 distinct patterns were identified. DNA sequences of representative clones from each digestion pattern were determined by dideoxynucleotide sequencing.

[0127] Plasmids from individual clones exhibiting positive binding to IGF-IR and unique DNA profile were used to transform a nonsuppressor E.coli host HB2151. Expression of the Fab fragments in HB2151 was induced by culturing the cells in 2YTA medium containing 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG, Sigma) at 30°C. A periplasmic extract of the cells was prepared by resuspending the cell pellet in 25 mM Tris (pH 7.5) containing 20% (w/v) sucrose, 200 mM NaCl, 1 mM EDTA and 0.1 mM PMSF, followed by incubation at 4°C with gentle shaking for 1 h. After centrifugation at 15,000 rpm for 15 min, the soluble Fab protein was purified from the supernatant by affinity

chromatography using Protein G column followed the manufacturer's protocol (Amersham Pharmacia Biotech).

[0128] Candidate binding Fab clones were screened for competitive blocking of radiolabeled human IGF-I ligand to immobilized IGF-IR (100ng/well) coated onto 96 strip-well plates. Fab preparations were diluted and incubated with IGF-IR plates for 0.5-1 hour at room temperature in PBS/0.1%BSA. Forty 40 pM of ^{125}I-IGF-I was then added and the plates incubated an additional 90 minutes. Wells were then washed 3 times with ice-cold PBS/0.1% BSA, dried, and then counted in a gamma scintillation counter. Candidates that exhibited greater than 30% inhibition of control radiolabeled ligand binding in single point assay were selected and *in vitro* blocking titers determined. Four clones were identified. Of these, only Fab clone 2F8 was shown to inhibit ligand binding by more than 50%, with an IC_{50} of approximately 200nM, and it was selected for conversion to full length IgG1 format. The heavy chain variable region sequence and translated amino acid sequence for 2F8 is shown in Figures 1 and 2, respectively. The DNA sequence and translated polypeptide sequence of the 2F8 heavy chain engineered as full length IgG1 are shown in Figures 3 and 4, respectively.

[0129] Fab 2F8 sequencing determined that this Fab possessed a lambda light chain constant region. The DNA sequence and translated amino acid sequence of the 2F8 light chain are shown in Figures 5 and 6, respectively. The sequences for full-length lambda light chain format are shown in Figures 7 and 8. Binding kinetic analysis was performed on 2F8 IgG using a BIAcore unit. This antibody was determined to bind to the IGF-IR with an affinity of 0.5 –1 nM (0.5-1 x 10^{-9} M).

[0130] In order to improve the affinity of this antibody, a second generation Fab phage library was generated in which the 2F8 heavy chain was conserved and the light chain was varied to a diversity of greater than 10^8 unique species. This method is termed light chain shuffling and has been used successfully to affinity mature selected antibodies for a given target antigen (Chames et al., *J. Immunol.* 169:1110-18 (2002)). This library was then screened for binding to the human IGF-IR (10 μg/ml) following procedures as described above, and the panning process repeated an additional three rounds with reduced IGF-IR concentration (2 μg/ml) for enrichment of high affinity binding Fabs. Seven clones were analyzed following round four. All 7 contained the same DNA sequence and restriction digest profile. The single isolated Fab was designated A12 and shown to possess a lambda

light chain constant region. The light chain DNA sequence is shown in Figure 9 and amino acid sequence in Figure 10. Complete lambda light chain sequence and translated polypeptide sequence are shown in Figures 11 and 12, respectively. Amino acid sequence comparison of 2F8 and A12 light chains determined that the two variable regions differed by a total of 11 amino acids (refer to Figures 13 and 14). Nine of the changes were present within CDR regions, with the majority (6 amino acid residues) occurring within CDR3.

[0131] A comparison of the two antibody (full IgG) affinities for human IGF-IR and their ligand blocking activity is shown in Table 3. Binding results were determined by human IGF-IR ELISA and represent the concentration of titered antibody necessary to achieve 50% binding relative to saturation. Blocking results represent the level of antibody necessary to inhibit 50% binding of ^{125}I-IGF-I ligand to immobilized human IGF-IR. Affinity was determined by BIAcore analysis according to manufacturer's specifications (Pharmacia BIACORE 3000). Soluble IGF-IR was immobilized on the sensor chips and antibody binding kinetics determined.

Table 3 - Antibody binding characteristics

Antibody	Binding (ED_{50})	Blocking (EC_{50})	Affinity
2F8	2.0 nM	3-6 nM	$K_D = 6.5 \times 10^{-10}$
			$K_{on} = 2.8 \times 10^{5}$
			$K_{off} = 1.8 \times 10^{-4}$
A12	0.3 nM	0.6-1nM	$K_D = 4.1 \times 10^{-11}$
			$K_{on} = 7.2 \times 10^{5}$
			$K_{off} = 3.0 \times 10^{-5}$

[0132] The antibody changes incurred in 2F8 light chain to generate antibody A12 effected a significantly higher affinity of A12 for IGF-IR than 2F8. Concomitantly, this increase effected a greater binding ability of A12 for the receptor, as determined by ELISA, and at least a three-fold increase in blocking activity of ligand for immobilized receptor. Figure 15 shows a representative titration of the two anti-IGF-IR antibodies in receptor blocking assay. The activity of A12 remained the same, irrespective of whether the light chain was engineered with a human lambda or kappa class constant region. Antibody A12 engineered with a lambda class light chain was utilized in all subsequent procedures. In this assay, A12 inhibited the binding of radiolabeled IGF-I to IGF-IR to a greater extent than competition with cold ligand. The activity of 2F8 was comparable to competition with cold

ligand. This is consistent with the relative affinities of the two antibodies (see Table 3) and IGF-I (0.5-1 nM).

Engineering and expression of fully human IgG1 anti-IGF-IR antibodies from Fab clones.

[0133] The DNA sequences encoding the heavy and light chain genes of Fabs 2F8 and A12 were amplified by polymerase chain reaction (PCR) using the Boerhinger Mannheim Expand kit according to manufacturer's instructions. Forward and reverse primers contained sequences for restriction endonuclease sites for cloning into mammalian expression vectors. The recipient vector for the heavy chain contained the entire human gamma 1 constant region cDNA sequence, flanked by a strong eukaryotic promoter and a 3' polyadenylation sequence. The full-length lambda light chain sequences for 2F8 or A12 were each cloned in to a second vector possessing only the eukaryotic regulatory elements for expression in mammalian cells. A selectable marker was also present on this vector for selection of stable DNA integrants following transfection of the plasmid into mammalian cells. Forward primers were also engineered with sequences encoding a strong mammalian signal peptide sequence for proper secretion of the expressed antibody. Following identification of properly cloned immunoglobulin gene sequences, the DNAs were sequenced and tested for expression in transient transfection. Transient transfection was performed into the COS7 primate cell line using Lipofection, according to manufacturer's specifications. At 24 or 48 hours post-transfection, the expression of full IgG antibody was detected in conditioned culture supernatant by anti-human-Fc binding ELISA. ELISA Plates (96 well) were prepared by coating with 100 ng/well of a goat-anti-human Fc-specific polyclonal antibody (Sigma) and blocked with 5% milk/PBS overnight at 4°C. The plates were then washed 5 times with PBS. Conditioned supernatant was added to wells and incubated for 1.5 hours at room temperature. Bound antibody was detected with a goat anti-human lambda light chain-HRP antibody (Sigma) and visualized with TMB reagents and microplate reader as described above. Large scale preparation of anti-IGF-IR antibodies was achieved by either large scale transient transfection into COS cells, by scale-up of the Lipofection method or by stable transfection into a suitable host cell such as a mouse myeloma cell line (NS0, Sp2/0) or a Chinese hamster ovary cell line (CHO). Plasmid encoding the anti-IGF-IR antibodies were transfected into host cells by electroporation and selected in appropriate drug selection medium for approximately two weeks. Stably selected colonies were screened for antibody

expression by anti-Fc ELISA and positive clones expanded into serum free cell culture medium. Antibody production from stably transfected cells was performed in suspension culture in spinner flasks or bioreactors for a period of up to two weeks. Antibody generated by either transient or stable transfection was purified by ProA affinity chromatography (Harlow and Lane. Antibodies. A Laboratory Manual. Cold Spring Harbor Press. 1988), eluted into a neutral buffered saline solution, and quantitated.

Determination of ligand blocking activity of anti-IGF-IR monoclonal antibodies on human tumor cells.

[0134] The anti-IGF-IR antibodies were then tested for blocking of radiolabeled ligand to native IGF-IR on human tumor cells. Assay conditions were performed according to Arteaga and Osborne (*Cancer Res.* 49:6237-41 (1989)), with minor modifications. MCF7 human breast cancer cells were seeded into 24 well dishes, and cultured overnight. Sub-confluent monolayers were washed 2-3 times in binding buffer (Iscove's Medium/0.1% BSA) and antibody added in binding buffer. After a short incubation with the antibody at room temperature, 40 pM ^{125}I-IGF-I (approximately 40,000 cpm/well) was added to each well and incubated for an additional hour with gentle agitation. The wells were then washed three times with ice-cold PBS / 0.1% BSA. Monolayers were then lysed with 200 µl 0.5N NaOH and counted in a gamma counter. The results are shown graphically in Figure 16. On human tumor cells, antibody A12 inhibited ligand binding to IGF-IR with an IC_{50} of 3 nM (0.45 µg/ml). This was slightly lower than the inhibitory activity of cold IGF-I ligand (IC_{50} = 1 nM), but better than the inhibitory activity of cold IGF-II (IC_{50} = 9 nM). The differences observed for the two IGF ligands can likely be attributed to the slower binding kinetics of IGF-II for the IGF-IR than ligand IGF-I (Jansson et al., *J. Biol. Chem.* 272:8189-97 (1997). The IC_{50} for antibody 2F8 was determined to be 30 nM (4.5 µg/ml). We subsequently determined the IGF-I ligand blocking activity of the A12 antibody on several different human tumor types. The results are shown in Table 4. Antibody A12 was effective in binding to endogenous cellular IGF-IR and inhibiting ligand binding to a range of human tumor types including cell lines from breast, pancreatic, and colorectal tissue.

Table 4. Inhibitory activity of antibody A12 on IGF-I binding to different human tumor types		
Cell line	Cell type	Blocking IC_{50}
MCF7	breast	3 nM
T47D	breast	6 nM
OV90	ovarian	6 nM
BXPC3	pancreatic	20 nM
HPAC	pancreatic	10 nM
HT-29	colorectal	10 nM
SK-ES1	Ewing sarcoma	2 nM
8226	myeloma	20 nM

[0135] The IGF-IR shares considerable homology with the insulin receptor (IR). To determine if the anti-IGF-IR antibodies were specific to this IGF-IR and did not block insulin binding, a cell-based blocking assay was performed on human ZR-75I breast cancer cells. Because insulin can bind to IGF-IR, albeit at three orders of magnitude lower affinity than for the IR, we utilized the human breast cancer line ZR-75I that possesses a higher IR to IGF-IR ratio in comparison to MCF7 cells. By using this line, we reasoned that insulin binding to the cells would be more indicative of specific IR binding. The assay was performed as described above for MCF7 cells and the results shown in Figure 17. Although cold insulin was able to titrate the binding of radiolabeled insulin to cells, neither 2F8 nor the high affinity A12 antibody blocked insulin binding, even at a concentration of 200 nM antibody, consistent with selective binding of these antibodies to IGF-IR and not IR.

Antibody-mediated inhibition of ligand-dependent cell mitogenesis.

[0136] In order to determine if blocking of IGF-I binding to IGF-IR inhibited cellular proliferation, a mitogenic assay was performed on MCF7 breast cancer cells, BxPC-3 pancreatic cancer cells and HT-29 colon cancer cells. The assay was performed according to Prager, et al. (*Proc. Natl. Acad. Sci. U.S.A.* 91:2181-85 (1994), with some modification. Cells were plated into 96-well tissue culture plates at 5000-10000 cells/well and allowed to adhere overnight. The medium was then replaced with serum free defined medium and incubated overnight at 37°C. Cells were then incubated with IGF-I with or without antibody A12 and incubated overnight at 37°C. 0.25 μCi [^{3}H]thymidine was then added to each well and incubated for 5 hours at 37°C. The supernatant was aspirated and the cells suspended by

trypsinization for 5 minutes. The cells were then collected onto a filter and washed three times with water, using a cell harvester. After drying, the filter was processed for reading in a scintillation counter. The results are shown in Figures 18A, B and C (MCF7, BxPC-3 and HT-29 respectively). IGF-I datapoints show titration of the ligand to determine the amount necessary to achieve the maximum mitogenic response. In measuring the activity of antibody A12 on various cancer cell types, IGF-I was added at a concentration of 5 nM and the antibody titered from 200 nM to 0.05 nM. Antibody A12 inhibited MCF7 mitogenesis in response to IGF-I ligand in a dose-dependent fashion, with an IC_{50} of 6 nM.

[0137] Antibody A12 was then tested for mitogenic inhibition on several additional human tumor cells lines and the results shown in Table 5. Antibody A12 was effective at inhibiting IGF-I ligand-mediated mitogenesis of a variety of human tumor cell lines, including breast cancer, colorectal cancer, and multiple myeloma.

Table 5. Inhibitory activity of antibody A12 on mitogenesis of different human tumor cell lines

Cell line	Cell type	IC_{50}
MCF7	breast	6 nM
T47D	breast	7 nM
BT474	breast	5 nM
BXPC3	pancreatic	2 nM
HT-29	colorectal	6 nM
SK-ES1	Ewing sarcoma	10 nM
8226	myeloma	5 nM

Antibody-mediated inhibition of IGF-I directed receptor phosphorylation and downstream signaling.

[0138] To visualize the inhibitory effect of the anti-IGF-IR antibodies on IGF-I signaling, receptor auto-phosphorylation and downstream effector molecule phosphorylation analysis was performed in the presence or absence of antibody A12 or 2F8. The MCF7 human breast cancer cell line was selected for use due to its high IGF-IR density. Cells were plated into 10 cm or 6 well culture dishes and grown to 70-80% confluence. The monolayers were then washed twice in PBS and cultured overnight in serum free defined medium. Anti-IGF-IR antibody was then added in fresh serum-free media (100 nM-10 nM) and incubated

cells 30 minutes before addition of ligand (10 nM). Cells were incubated with ligand for 10 minutes, then placed on ice and washed with ice-cold PBS. The cells were lysed by the addition of lysis solution (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% TritonX-100, 1 mM EDTA, 1 mM PMSF, 0.5 mM Na_3VO_4, 1 μg/ml leupeptin, 1 μg/ml pepstatin, and 1 μg/ml aprotinin) and the cells scraped into a centrifuge tube kept on ice for 15 minutes. The lysate was then clarified by centrifugation at 4°C. Solubilized IGF-IR was then immunoprecipitated (IP) from the lysate. Antibody 3B7 (Santa Cruz) or A12 at 1 μg/ml were incubated with 400 μl of lysate overnight at 4°C. Immune complexes were then precipitated by the addition of ProA-sepharose beads for 2 hours at 4°C, pelleted, and washed 3 times with lysis buffer. IPs bound to the ProA beads were stripped into denaturing gel running buffer. Lysate or IP were processed for denaturing gel electrophoresis and run on a 4-12% acrylamide gel and blotted to nylon or nitrocellulose membrane by western blot according to Towbin et al. (*Biotechnology* 24:145-9 (1992)). Tyrosine phosphorylated protein was detected on the blot using an anti-p-tyrosine antibody (Cell Signaling #9411) and an anti-mouse-HRP secondary antibody. IGF-IR was detected with monoclonal antibody C-20 (Santa Cruz). For Akt phosphorylation, phospho-Akt was detected with antibody #559029 and total Akt with #559028 (BD Pharmingen). For MAPK phosphorylation, phospho-p44/42 was detected with #9101 and total p44/42 with #9102 (Cell Signaling Tech.). Bands were visualized with the ECL reagent on X-ray film.

[0139] As shown in Figure 19A, auto-phosphorylation of the IGF-IR in MCF7 cells was arrested following serum deprivation, and the addition of either 2F8 or A12 alone did not induce receptor phosphorylation, thereby demonstrating a lack of detectable agonist activity. Upon the addition of 10 nM IGF-I, IGF-IR phosphorylation was strongly induced. Antibody 2F8 effected an approximately 50% reduction in IGF-IR phosphorylation, whereas the high affinity antibody A12 nearly completely blocked phosphorylation. Similarly, antibody A12 inhibited auto-phosphorylation of IGF-IR in HT-29 colorectal and BxPC-3 pancreatic cancer cells (Fig. 19B).

[0140] Downstream effector signaling in response to IGF-I was also inhibited by the anti-IGF-IR antibodies (Fig. 20). MAPK phosphorylation was considerably inhibited by both 2F8 and A12. Phosphorylation of the anti-apoptotic molecule Akt was less sensitive to anti-IGF-IR antibody blockade with 2F8. It effected only a slight reduction in Akt phosphorylation. A12 significantly inhibited Akt phosphorylation, even at a concentration of

10nM. Antibody A12 was equally proficient in immunoprecipitating solubilized IGF-IR as the commercial antibody 3B7, but A12 was not capable of detecting denatured IGF-IR immobilized on nylon membranes following western blot transfer.

FACs binding analysis of monoclonal antibody A12 to tumor cell lines.

[0141] Since A12 was capable of immunoprecipitating endogenous IGF-IR, we were therefore interested in determining if A12 could also be used as detection antibody for fluorescence activated cell sorting (FACs). Human tumor cell lines were grown in culture, scraped into ice-cold PBS, and counted. Primary antibody, A12 (0.5μg), was added to approximately 5 million cells in 250 μl PBS/5%FBS and incubated on ice for 1 hour. The cells were then diluted to 3 mls in PBS/5% FBS, pelleted, and the supernatant aspirated. Secondary phycoerythin (PE) -labeled goat anti-human IgG $F(ab)_2$ fragment was then added in 250 μl PBS/5% FBS at 1:200 and incubated on ice for 60 minutes. Afterwards, the cells were again diluted and pelleted, as before, then resuspended in 500 μl PBS/5% FBS. FACs analysis was then performed on a Epics XL unit (Coulter). As shown in Fig. 21, antibody A12 fully shifted the human breast cancer cell line MCF7 and the human leukemia cell line HEL. IGF-IR negative mouse embryo fibroblasts (R- cells) (obtained from R. Baserga, Thomas Jefferson University, Philadelphia, PA) served as the negative control. A12 failed to bind to these cells, indicative of antibody binding specificity for the IGF-IR. A12 did, however, bind and partially shift the mouse tumor cell line Lewis Lung carcinoma, suggesting that this anti-human IGF-IR antibody possesses some cross-reactivity for the mouse IGF-IR.

IGF-I receptor internalization following binding of antibody A12.

[0142] Antibody A12 has been shown to bind native IGF-IR on human tumor cells with high affinity. Antibody A12 was radio-iodinated with ^{125}iodine using IODO-beads (Pierce) according to manufacturer's instructions. MCF7 human breast cancer cells were plated into 6-well plates and cultured overnight to 50% confluence. One microgram of ^{125}I-A12 was added to each well and incubated at 37°C or kept on ice at 4°C. Plates were incubated for 30 minutes, 90 minutes, or 180 minutes and each time point performed in triplicate. The culture at 4°C was held for 180 minutes. At each time point, wells were washed 1x w/PBS, then stripped for 5 minutes with 100mM glycine-HCl, 2M urea, pH 2.5. The stripped material, representing membrane bound antibody was kept on ice for counting.

Wells were then washed 3 times with PBS and cells solubilized with 1N NaOH/1% TritonX100. The solubilized fraction represented the internalized antibody. Stripped and solublized fractions were then read on a gamma counter and plotted with standard deviation. As shown in Fig. 22, the level of internalized radioactivity increased with time in the cells cultured at 37°C, while little uptake was observed in cells maintained at 4°C where membrane transport should be severely retarded. This demonstrated that, upon binding to the IGF-IR, antibody A12 is rapidly internalized, potentially leading to a depletion of surface bound receptor.

[0143] IGF-IR surface receptor density was determined by FACS. Adherent MCF 7 cells were treated for 4 h with 50 nM of antibody A12 of IGF-I at 37°C. Cells were washed in ice-cold PBS/5% BSA twice, and 1 x 10^6 cells were aliquoted to staining tubes and placed on ice. An IGF-IR alpha-polypeptide specific, non-blocking mouse monoclonal antibody (Ab-1, NeoMarkers, Fremont, CA), was then incubated with cells at 4°C for 2 h. After PBS/BSA washes, cells were incubated with anti-mouse IgG phycoerythrin-conjugated secondary antibody (PharMingen, BD Biosciences) for 1 h on ice. After PBS/BSA wash, cells were analyzed by fluorescence-activated cell-sorting assay using a FACSvantage SE flow cytometer (BD Bioscience). As shown in Fig. 22b, exposure to A12 resulted in a significant reduction in the fluorescence intensity of MCF7 cells, indicative of surface receptor down-modulation due to internalization. Calculated mean fluorescence intensity ratio indicated a reduction in IGF-IR surface staining of 90% after incubation with A12. This shift was not seen when cells were incubated with A12 at 4°C, consistent with an energy-dependent, antibody-mediated, receptor internalization process. Exposure of cells to IGF-I did not cause a significant change in surface IGF-IR fluorescence intensity, consistent with Western blot analysis showing little effect of ligand on IGF-IR degradation

[0144] Total cellular IGF-IR was determined HT-29 cells and BxPC-3 cells in response to treatment with IGF-I or A12. As shown in Fig. 22c, addition of IGF-I to growing cultures had no effect on IGF-IR expression. In contrast, addition of antibody A12 resulted in severe depletion of IGF-IR levels in the cells after 3-6 hours.

Growth inhibition of human colorectal tumors alone or in combination with irinotecan (CPT-11).

[0145] We were interested to determine if the anti-IGF-IR antibodies were capable of inhibiting human tumor growth *in vivo* in a nude mouse xenograft model. Tumors were induced in 3-4 week old athymic nude (nu/nu) mice by subcutaneous injection of 2-3 million viable HT-29 human colorectal cancer cells in cell culture medium. The tumors were allowed to establish and antibody treatment started when the tumor volume reached 200 mm^3. Ten animals were injected with tumor cells per treatment group. Antibody was injected intraperitoneally (IP) every three days at 1 mg or 0.5 mg in 0.5 ml TBS. The drug irenotecan (CPT-11) (LKT Laboratories) was injected IP (100 mg/kg) once a week for four weeks from the initiation of antibody treatment. Control animals received a class matched irrelevant human IgG antibody. Tumor measurements were performed at regular intervals using Vernier calipers, measuring height, width, and length and calculated to determine the total tumor volume. The study was terminated when control tumors reached 3000 mm^3. As shown in Fig. 23, doses of antibody 2F8 at either 0.5 mg or 1 mg every three days effected a significant inhibition ($P < 0.05$) of tumor growth in this model. There was no statistical difference between the tumor sizes from groups treated with 0.5 or 1 mg 2F8 and the responses were similar to treatment with CPT-11 alone. When 2F8 and CPT-11 were given together the combination resulted in greater inhibition of tumor growth (72% decrease), demonstrating that anti-IGF-IR therapy could enhance the anti-tumor activity of the chemotherapeutic agent CPT-11 on tumor growth.

Anti-tumor activity of antibody A12 on human colorectal tumors in vivo.

[0146] Antibody A12 possesses a 10-fold higher affinity for the IGF-IR than antibody 2F8. Since significant tumor inhibition was observed in vivo with antibody 2F8, we investigated the activity of A12 on the growth of the human colorectal cancer line HT-29 in a mouse xenograft model. Tumors were induced as previously described, and antibody treatment initiated once tumors were established (200 mm^3 size). Antibody treatment was then given at a concentration of 1 mg, 100 μg, or 10 μg every three days throughout the duration of the experiment. Ten animals were used per treatment group and control animals received a class match IgG control antibody. As shown in Fig. 24, antibody A12 effected a 74% reduction in tumor growth compared to control ($P < 0.05$). This demonstrated that A12

was effective as a single therapeutic at inhibiting colorectal tumor growth in this xenograft model. A clear dose-response effect was noted in this experiment. Anti-tumor activity was also observed with a dose of 100 μg A12.

Activity of antibody A12 on human breast cancer in vivo *in a xenograft tumor model.*

[0147] Antibody A12 exhibited strong inhibitory activity on the IGF-dependent mitogenic stimulation and proliferation of MCF7 cells in vitro. In order to assess its activity on MCF7 tumor growth in vivo, a mouse xenograft tumor model was utilized. MCF7 cells were originally isolated from an estrogen-dependent human tumor and require exogenously added estrogen for maintenance and growth in vivo. Nude mice were implanted with biodegradable estrogen pellets (0.72mg 17-β -estradiol/pellet, 60 day release). In addition, at the time of subcutaneous tumor cell injection, the mice were also injected in the right flank subcutaneously with 0.5 mg of estradiol in a 50 μl suspension of sesame seed oil. Tumors were allowed to establish a size of approximately 150 mm^3 before antibody treatment was initiated. Antibody was injected at 1 mg, 100 μg, and 10 μg doses every three days and continued for the duration of the experiment. At 29 days, treatment of animals with 1 mg of A12 effected an 89% reduction in tumor growth (Fig. 25). Minimal growth was apparent for the established tumors in this treatment group. A dose dependent response was noted for A12 treatments in this model. The study demonstrated that A12 was effective in significantly reducing the growth of a human breast cancer cell line *in vivo*. Further, treating with antibody alone at 1 mg/dose, tumor regression was observed and continued to the termination of the study (50 days).

Efficacy of A12 in combination with CPT-11 or gemcitabine in BxPC-3 pancreatic carcinoma xenografts

[0148] Athymic nu/nu mice were injected subcutaneously with 2 x 10^6 BxPC-3 human pancreatic carcinoma cells mixed 1:1 with Matrigel. Twenty days later, when tumors reached 200-300mm^3, mice were randomized and divided into treatment groups: 1) TBS control; 2) mAb A12 at 1mg/dose, 3 times per week; 3) 1 mg irinotecan, once every 7 days; 4) mAb A12 + irinotecan; 5) 2.5 mg gemcitabine, once every 7 days; 6) mAb A12 + gemcitabine. All treatments were administered by intraperitoneal injection. Tumor measurements were recorded twice weekly using the formula $Volume = (\pi/6)\ l \times w^2$.

[0149] At day 18 three animals per group were sacrificed and the tumors resected for midpoint histological evaluation. Treatment and tumor measurements continued on the remaining animals until day 44 with the exception of the TBS group. This control group was sacrificed at day 37 due to tumor ulceration and necrosis. At termination of the study final tumor measurements were recorded, animals sacrificed, and four per group had tumors resected for endpoint histological evaluation.

[0150] BxPC-3 tumors were very responsive to mAb A12, and 2 of eight animals had partial tumor regressions after five weeks of treatment. The response of BxPC-3 tumors to irinotecan or gemcitabine alone was comparable to the antibody, but there were no tumor regressions. Antibody A12, when combined with irinotecan or gemcitabine was more effective than any agent alone (Fig. 26), with irinotecan + Mab A12 being the more effective combination. For combined A12 and irinotecan, three of nine animals had partial tumor regressions. However, there were no tumor regressions among the animals given irinotecan. (Table 5).

Table 5. Inhibition of Growth of BxPC-3 Xenografts

	mAb A12		Irinotecan		mAb A12 + Irinotecan		Gemcitabine		mAb A12 + Gemcitabine	
Day	T/C %	Regressions*	T/C %	Regressions	T/C %	Regressions	T/C %	Regressions	T/C %	Regressions
19	57	2 /12	52	0 /12	40	3 /12	68	0 /11	44	0 /12
23	49	2 / 9	45	0 / 8	32	3 / 9	65	0 / 8	42	1 / 9
26	40	2 / 9	35	0 / 8	26	3 / 9	58	0 / 8	37	1 / 9
30	40	2 / 8	34	0 / 8	22	3 / 9	56	0 / 8	36	1 / 9
32	39	2 / 8	34	0 / 8	19	3 / 9	48	0 / 8	31	1 / 9
37	30	2 / 8	26	0 / 8	15	3 / 9	37	0 / 8	24	1 / 9
41	-	2 / 8	-	0 / 7	-	3 / 8		0 / 8	-	1 / 8
44	-	2 / 8	-	0 / 7	-	3 / 8		0 / 7	-	0 / 6

T/C % is tumor growth inhibition relative to control

*Regressions defined as individual tumor volumes < day 1 of treatment.

Efficacy of A12 in combination with CPT-11 or paclitaxel in HT-29 colorectal cancer xenografts

[0151] Female thymic nu/nu mice were injected subcutaneously with HT-29 human colon carcinoma cell suspension, at 5 x 10^6 cells in 0.4 ml mixed 1:1 with Matrigel. When tumors reached ~200mm^3 mice were randomized and divided into treatment groups: 1) TBS control; 2) mAb A12 at 1mg/dose, 3 times per week; 3) 2 mg irinotecan, once every 7 days; 4) mAb A12 + irinotecan; 5) 121 μg paclitaxel in 0.2 ml, once evrey 7 days; 6) mAb A12 + paclitaxel. All treatments were administered by intraperitoneal injection.

[0152] Treatment continued for six weeks and tumor measurements were recorded twice weekly using the formula $Volume = (\pi/6)\ l\ x\ w^2$. At day 21 four animals per group were sacrificed and the tumors resected for histological evaluation. Treatment and tumor measurements continued on the remaining animals until day 40. At that time final tumor measurements were recorded, animals sacrificed, and four per group had tumor resected for endpoint histological evaluation.

[0153] Single-agent mAb A12, CPT-11, or paclitaxel significantly ($P < 0.02$) inhibited the growth of HT-29 xenografts compared to the TBS control group (Fig. 27). Combination therapy of mAb A12 with either CPT-11 or paclitaxel showed a significant inhibition ($P < 0.003$) in tumor growth compared to either treatment alone.

[0154] No tumor regressions were observed in the single-therapy groups. Control tumors *in situ* appeared highly vascularized at day 22 as did single-therapy groups at day 40. However, combination therapy with CPT-11 + mAb A12 resulted in partial tumor regressions in six out of eight animals, and combination therapy with paclitaxel and mAb A12 resulted in regressions in three of eight animals.

Table 6. Inhibition of Growth of HT-29 Xenografts

	mAb A12	CPT-11	paclitaxel	A12 + CPT-11		A12 + paclitaxel	
Day	T/C %	T/C %	T/C %	T/C %	Regressions*	T/C %	Regressions
12	69	63	66	52	0 / 12	48	1 /12
15	62	59	59	42	0 / 12	40	3 /12
19	64	61	62	30	3 / 12	32	5 /12
22	58	52	56	24	4 / 8	28	4 / 8
26	62	47	54	17	6 / 8	27	3 / 8
29	61	45	55	14	6 / 8	24	3 / 8
33	58	40	52	11	5 / 8	22	3 / 8
36	53	40	45	9	5 / 8	22	2 / 8
40	54	41	52	10	2 / 6	26	2 / 8

T/C % is tumor growth inhibition relative to control
*Regressions defined as individual tumor volumes < day 1 of treatment.

[0155] It is understood and expected that variations in the principles of invention herein disclosed may be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

What is claimed is:

1. An isolated human antibody or fragment thereof that specifically binds to insulin-like growth factor-I receptor (IGF-IR) and has at least one property selected from the group consisting of

(i) inhibits binding of IGF-I or IGF-II to IGF-IR;

(ii) neutralizes activation of IGF-IR by IGF-I or IGF-II;

(iii) reduces IGF-IR surface receptor by at least about 80%; and

(iv) binds to IGF-IR with a K_d of about 3×10^{-10} M^{-1} or less.

2. The antibody or antibody fragment of Claim 1, which has all of said properties.

3. The antibody or antibody fragment of Claim 1, wherein the antibody or antigen binding fragment thereof reduces surface IGF-IR by at least about 85%.

4. The antibody or antibody fragment of Claim 1, wherein the antibody or antigen binding fragment thereof reduces surface IGF-IR by at least about 90%.

5. The antibody or antibody fragment of Claim 1, which binds to IGF-IR with a K_d of about 1×10^{-10} M^{-1} or less.

6. The antibody or antibody fragment of Claim 1, which binds to IGF-IR with a K_d of about 5×10^{-11} M^{-1} or less.

7. The antibody or antibody fragment of Claim 1, which inhibits phosphorylation of a downstream substrate of IFG-IR.

8. The antibody or antibody fragment of Claim 7, wherein the downstream substrate is selected from the group consisting of MAPK, Akt, and IRS-2, and phosphorylation is inhibited by about 50% or more.

9. The antibody or antibody fragment of Claim 1, which promotes tumor regression *in vivo*.

10. The antibody or antibody fragment of Claim 1, which promotes tumor regression *in vivo* when administered with an anti-neoplastic agent.

11. The antibody or antibody fragment of Claim 1, which competes for binding to IGF-IR with an antibody selected from the group consisting of

the antibody having a heavy chain variable domain represented by SEQ ID NO:2 and a light chain variable domain represented by SEQ ID NO:6; and

the antibody having a heavy chain variable domain represented by SEQ ID NO:2 and a light chain variable domain represented by SEQ ID NO:10.

12. The antibody or antibody fragment of Claim 1, which specifically binds to insulin-like growth factor-I receptor (IGF-IR) and comprises at least one complementarity-determining region (CDR) having an amino acid sequence selected from SEQ ID NO:13 at V_HCDR1, SEQ ID NO:15 at V_HCDR2, SEQ ID NO:17 at V_HCDR3, SEQ ID NO 19 at V_LCDR1, SEQ ID NO:21 at V_LCDR2, SEQ ID NO:23 at V_LCDR3, SEQ ID NO 25 at V_LCDR1, SEQ ID NO:27 at V_LCDR2, and SEQ ID NO:29 at V_LCDR3.

13. The antibody or antigen binding fragment of Claim 1, which comprises SEQ ID NO:13 at V_HCDR1, SEQ ID NO:15 at V_HCDR2, and SEQ ID NO:17 at V_HCDR3.

14. The antibody or antigen binding fragment of Claim 1, which comprises SEQ ID NO 19 at V_LCDR1, SEQ ID NO:21 at V_LCDR2, and SEQ ID NO:23 at V_LCDR3.

15. The antibody or antigen binding fragment of Claim 1, which comprises SEQ ID NO 25 at V_LCDR1, SEQ ID NO:27 at V_LCDR2, and SEQ ID NO:29 at V_LCDR3.

16. The antibody of Claim 1, wherein the heavy chain variable domain has at least 90% sequence homology to SEQ ID NO:2.

17. The antibody of Claim 1, wherein the light chain variable domain has at least 90% sequence homology to SEQ ID NO:6.

18. The antibody of Claim 1, wherein the light chain variable domain has at least 90% sequence homology to SEQ ID NO:10.

19. An isolated nucleic acid encoding a polypeptide selected from the group consisting of:

SEQ ID NO:2 from about amino acid residue 1 to about amino acid residue 130;

SEQ ID NO:6 from about amino acid residue 1 to about amino acid residue 109; and

SEQ ID NO:10 from about amino acid residue 1 to about amino acid residue 109.

20. The isolated nucleic acid of Claim 19, selected from the group consisting of:
SEQ ID NO:1 from about nucleotide 1 to about nucleotide 390;
SEQ ID NO:5 from about nucleotide 1 to about nucleotide 327; and
SEQ ID NO:9 from about nucleotide 1 to about nucleotide 327.

21. A recombinant vector comprising a nucleic acid of Claim 19.

22. A host cell comprising the vector of Claim 21.

23. A pharmaceutical composition comprising the antibody or antibody fragment of any one of Claims 1 to 18 and a pharmaceutically acceptable carrier.

24. A conjugate comprising the antibody or antibody fragment of any one of Claims 1 to 18 linked to a cytotoxic agent.

25. A conjugate comprising the antibody or antibody fragment of any one of Claims 1 to 18 linked to a label.

26. A therapeutic composition effective to inhibit growth of human tumor cells that express IGF-IR, which composition comprises the antibody or antigen binding fragment of any one of Claims 1 to 18.

27. The therapeutic composition of Claim 26, which further comprises an anti-neoplastic agent.

28. The therapeutic composition of Claim 27, wherein the anti-neoplastic agent is an inhibitor of topoisomerase I or topoisomerase II.

29. The therapeutic composition of Claim 27, wherein the anti-neoplastic agent is selected from the group consisting of irinotecan, camptothecan, and etoposide.

30. A therapeutic composition effective to promote regression of human tumors that express IGF-IR, which composition comprises the antibody or antibody fragment of any one of Claims 1 to 18.

31. The therapeutic composition of Claim 30, which further comprises an anti-neoplastic agent.

32. The therapeutic composition of Claim 31, wherein the anti-neoplastic agent is an inhibitor of topoisomerase I or topoisomerase II.

33. The therapeutic composition of Claim 31, wherein the anti-neoplastic agent is selected from the group consisting of irinotecan, camptothecan, or etoposide.

34. A method of neutralizing the activation of IGF-IR, which comprises administering to a mammal an effective amount of the antibody or antibody fragment of any one of Claims 1 to 18.

35. A method of treating a proliferative disorder comprising the step of administering an effective amount of the antibody or antibody fragment of any one of Claims 1 to 18.

36. The method of Claim 35, wherein the proliferative disorder is selected from the group consisting of acromegaly, retinal neovascularization, and psoriasis.

37. A method of inhibiting the growth of a cell that expresses IGF-IR, which comprises contacting the cell with an effective amount of the antibody or antibody fragment of any one of Claims 1 to 18.

38. The method of Claim 35, which further comprises contacting the cell with an effective amount of an anti-neoplastic agent.

39. The method of Claim 38, wherein the anti-neoplastic agent is an inhibitor of topoisomerase I or topoisomerase II.

40. The method of Claim 38, wherein the anti-neoplastic agent is selected from the group consisting of irinotecan, camptothecan, and etoposide.

41. A method of reducing tumor growth which comprises administering to a mammal an effective amount of the antibody or antibody fragment of any one of Claims 1 to 18.

42. The method of Claim 41, which further comprises administering an effective amount of an anti-neoplastic agent.

43. The method of Claim 42, wherein the anti-neoplastic agent is an inhibitor of topoisomerase I or topoisomerase II.

44. The method of Claim 42, wherein the anti-neoplastic agent is selected from the group consisting of irinotecan, camptothecan, and etoposide.

45. A method of promoting tumor regression which comprises administering to a mammal an effective amount of the antibody or antibody fragment of any one of Claims 1 to 18.

46. The method of Claim 45, which further comprises administering an effective amount of an anti-neoplastic agent.

47. The method of Claim 46, wherein the anti-neoplastic agent is an inhibitor of topoisomerase I or topoisomerase II.

48. The method of Claim 46, wherein the anti-neoplastic agent is selected from the group consisting of irinotecan, camptothecan, and etoposide.

49. The method of any one of Claims 41 to 48, wherein the tumor is a breast tumor, colorectal tumor, pancreatic tumor, ovarian tumor, lung tumor, prostate tumor, bone or soft tissue sarcoma or myeloma.

50. A method of inhibiting the growth of a cell that expresses IGF-IR, which comprises contacting the cell with an effective amount of an agent that is an inhibitor of topoisomerase I or topoisomerase II and an antibody or antigen binding fragment thereof that specifically binds to IGF-IR and has at least one property selected from the group consisting of

(i) inhibits binding of IGF-I or IGF-II to IGF-IR;
(ii) neutralizes activation of IGF-IR by IGF-I or IGF-II;
(iii) reduces IGF-IR surface receptor; and
(iv) binds to IGF-IR with a K_d of about 1×10^{-10} M^{-1} or less.

51. A method of reducing growth of a tumor that expresses IGF-IR, which comprises contacting the cell with an effective amount of an agent that is an inhibitor of topoisomerase I or topoisomerase II and an antibody or antigen binding fragment thereof that specifically binds to IGF-IR and has at least one property selected from the group consisting of

(i) inhibits binding of IGF-I or IGF-II to IGF-IR;
(ii) neutralizes activation of IGF-IR by IGF-I or IGF-II;
(iii) reduces IGF-IR surface receptor by at least about 80%; and
(iv) binds to IGF-IR with a K_d of about 1×10^{-10} M^{-1} or less.

52. A method of promoting regression of a tumor that expresses IGF-IR, which comprises contacting the cell with an effective amount of an agent that is an inhibitor of topoisomerase I or topoisomerase II and an antibody or antigen binding fragment thereof that specifically binds to IGF-IR and has at least one property selected from the group consisting of

(i) inhibits binding of IGF-I or IGF-II to IGF-IR;
(ii) neutralizes activation of IGF-IR by IGF-I or IGF-II;
(iii) reduces IGF-IR surface receptor by at least about 80%; and
(iv) binds to IGF-IR with a K_d of about 1×10^{-10} M^{-1} or less.

53. The method of any one of Claims 50 to 52, wherein the agent is selected from the group consisting of irinotecan, camptothecan, and etoposide.

54. The method of any one of Claims 50 to 52, wherein the antibody or antibody fragment is human.

55. The method of any one of Claims 50 to 52, wherein the antibody or antibody fragment is humanized.

56. The method of any one of Claims 51 and 52, wherein the tumor is a breast tumor, colorectal tumor, pancreatic tumor, ovarian tumor, lung tumor, prostate tumor, bone or soft tissue sarcoma or myeloma.

Figure 1

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GAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTC      50
GGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTA     100
TCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGATGGGAGGG     150
ATCATCCCTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGGCAG     200
AGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGCTGA     250
GCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGCGCCA     300
TTACGATTTTTGGAGTGGTCCACCCAAGACCACTACTACTACTACTACAT     350
GGACGTCTGGGGCAAAGGGACCACGGTCACCGTCTCAAGC               390
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Figure 2

EVQLVQSGAEVKKPGSSVKVSCKASGGTFS**SYAIS**WVRQAPGQGLEWMGG 50
IIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAP 100
LRFLEWSTQDHYYYYYMDVWGKGTTVTVSS 130

Figure 3

ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGT 50
ACATTCAGAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTG 100
GGTCCTCGGTGAAGGTCTCCTGCAAGGCTTCTGGAGGCACCTTCAGCAGC 150
TATGCTATCAGCTGGGTGCGACAGGCCCCTGGACAAGGGCTTGAGTGGAT 200
GGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCACAGAAGTTCC 250
AGGGCAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATG 300
GAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAG 350
AGCGCCATTACGATTTTTGGAGTGGTCCACCCAAGACCACTACTACTACT 400
ACTACATGGACGTCTGGGGCAAAGGGACCACGGTCACCGTCTCAAGCGCC 450
TCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCAC 500
CTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG 550
AACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC 600
ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGT 650
GGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACG 700
TGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAA 750
TCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCT 800
GGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCA 850
TGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC 900
GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA 950
TAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGG 1000
TGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAG 1050
TACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAAC 1100
CATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGC 1150
CCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTG 1200
GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG 1250
GCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACG 1300
GCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG 1350
CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCA 1400
CTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA 1440

Figure 4

MGWSCIILFLVATATGVHSEVQLVQSGAEVKKPGSSVKVSCKASGGTFSS 50
YAISWVRQAPGQGLEWMG**GIIPIFGTANYAQKFQG**RVTITADKSTSTAYM 100
ELSSLRSEDTAVYYCAR**APLRFLEWSTQDHYYYYYMDV**WGKGTTVTVSSA 150
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH 200
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK 250
SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH 300
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE 350
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL 400
VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ 450
QGNVFSCSVMHEALHNHYTQKSLSLSPGK 479

Figure 5

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TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGAC      50
AGTCAGGATCACATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCT     100
GGTACCAGCAGAAGCCAGGACAGGCCCCTGTACTTGTCATCTATGGTAAA     150
AACAACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGG     200
AAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATGAGGCTG     250
ACTATTACTGTAACTCCCGGGACAACAGTGATAACCGTCTGATATTTGGC     300
GGCGGGACCAAGCTGACCGTCCTCAGT                            327
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Figure 6

SSELTQDPAVSVALGQTVRITC**QGDSLRSYYAS**WYQQKPGQAPVLVIY**GK** 50
NNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYC**NSRDNSDNRLI**FG 100
GGTKLTVLS 109

Figure 7

ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGT 50
ACATTCATCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGG 100
GACAGACAGTCAGGATCACATGCCAAGGAGACAGCCTCAGAAGCTATTAT 150
GCAAGCTGGTACCAGCAGAAGCCAGGACAGGCCCCTGTACTTGTCATCTA 200
TGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCA 250
GCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT 300
GAGGCTGACTATTACTGTAACTCCCGGGACAACAGTGATAACCGTCTGAT 350
ATTTGGCGGCGGGACCAAGCTGACCGTCCTCAGT*CAGCCCAAGGCTGCCC* 400
CCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAG 450
GCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGT 500
GGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA 550
CACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGC 600
CTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCAC 650
GCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTGCAGAATGCTCTT 700
GA 702

Figure 8

MGWSCIILFLVATATGVHSSSELTQDPAVSVALGQTVRITC**QGDSLRSYY** 50
ASWYQQKPGQAPVLVIY**GKNNRPS**GIPDRFSGSSSGNTASLTITGAQAED 100
EADYYC**NSRDNSDNRLI**FGGGTKLTVLS*QPKAAPSVTLFPPSSEELQANK* 150
ATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLS 200
LTPEQWKSHRSYSCQVTHEGSTVEKTVAPAECS 233

Figure 9

```
TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGAC      50
AGTCAGGATCACATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAACCT     100
GGTACCAGCAGAAGCCAGGACAGGCCCCTATTCTTGTCATCTATGGTGAA     150
AATAAGCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGG     200
AAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCAGAAGATGAGGCTG     250
ACTACTATTGTAAATCTCGGGATGGCAGTGGTCAACATCTGGTGTTCGGC     300
GGAGGGACCAAGCTGACCGTCCTAGGT                            327
```

Figure 10

SSELTQDPAVSVALGQTVRITC**QGDSLRSYYAT**WYQQKPGQAPILVIY**GE** 50
NKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYC**KSRDGSGQHLV**FG 100
GGTKLTVLG 109

Figure 11

ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGT 50
ACATTCATCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGG 100
GACAGACAGTCAGGATCACATGCCAAGGAGACAGCCTCAGAAGCTATTAT 150
GCAACCTGGTACCAGCAGAAGCCAGGACAGGCCCCTATTCTTGTCATCTA 200
TGGTGAAAATAAGCGGCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCA 250
GCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCAGAAGAT 300
GAGGCTGACTACTATTGTAAATCTCGGGATGGCAGTGGTCAACATCTGGT 350
GTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAGCCCAAGGCTGCCC 400
CCTCGGTCACTCTGTTCCCGCCCTCCTCTGAGGAGCTTCAAGCCAACAAG 450
GCCACACTGGTGTGTCTCATAAGTGACTTCTACCCGGGAGCCGTGACAGT 500
GGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCA 550
CACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGC 600
CTGACGCCTGAGCAGTGGAAGTCCCACAGAAGCTACAGCTGCCAGGTCAC 650
GCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCTGCAGAATGCTCTT 700
GA 702

Figure 12

MGWSCIILFLVATATGVHSSSELTQDPAVSVALGQTVRITCQGDSLRSYY 50
ATWYQQKPGQAPILVIYGENKRPSGIPDRFSGSSSGNTASLTITGAQAED 100
EADYYCKSRDGSGQHLVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANK 150
ATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLS 200
LTPEQWKSHRSYSCQVTHEGSTVEKTVAPAECS 233

Figure 13

Heavy chain CDR1	CDR2	CDR3	
SYAIS	GIIPIFGTANYAQKFQG	APLRFLEWSTQDHYYYYYMDV	2F8/A12

Light chain CDR1	CDR2	CDR3	
QGDSLRSYYAS	GKNNRPS	NSRDNSDNRLI	2F8
QGDSLRSYYAT	GENKRPS	KSRDGSGQHLV	A12

Figure 14

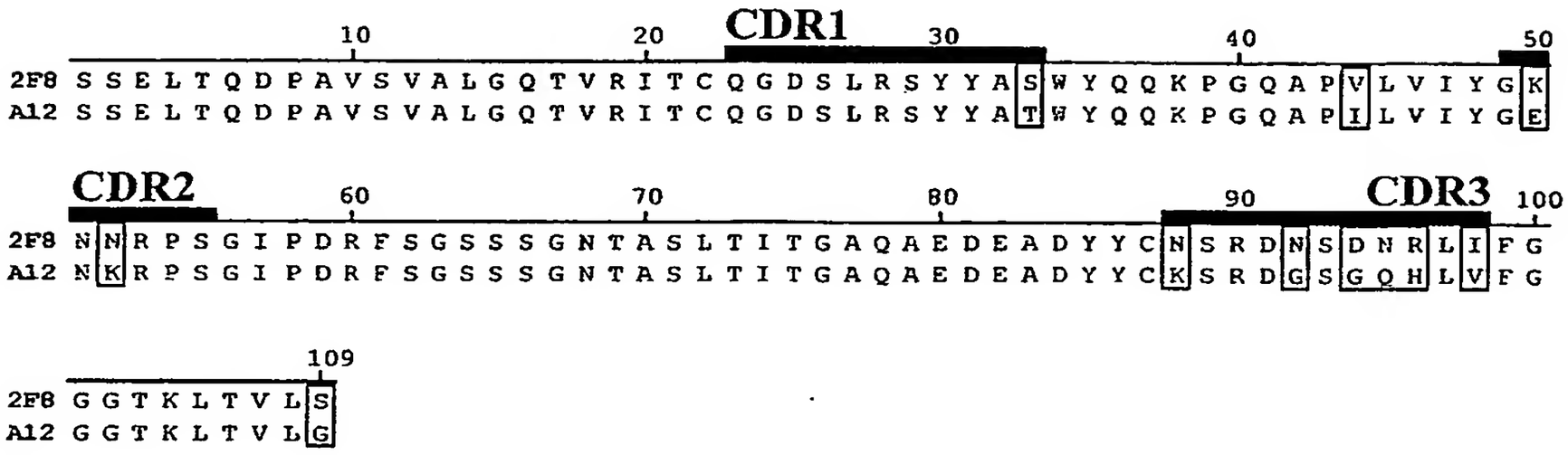

CDR1
CDR2
CDR3
10
20
30
40
50
60
70
80
90
100
109
2F8 S S E L T Q D P A V S V A L G Q T V R I T C Q G D S L R S Y Y A S W Y Q Q K P G Q A P V L V I Y G K
A12 S S E L T Q D P A V S V A L G Q T V R I T C Q G D S L R S Y Y A T W Y Q Q K P G Q A P I L V I Y G E
2F8 N N R P S G I P D R F S G S S S G N T A S L T I T G A Q A E D E A D Y Y C N S R D N S D N R L I F G
A12 N K R P S G I P D R F S G S S S G N T A S L T I T G A Q A E D E A D Y Y C K S R D G S G Q H L V F G
2F8 G G T K L T V L S
A12 G G T K L T V L G

Figure 15

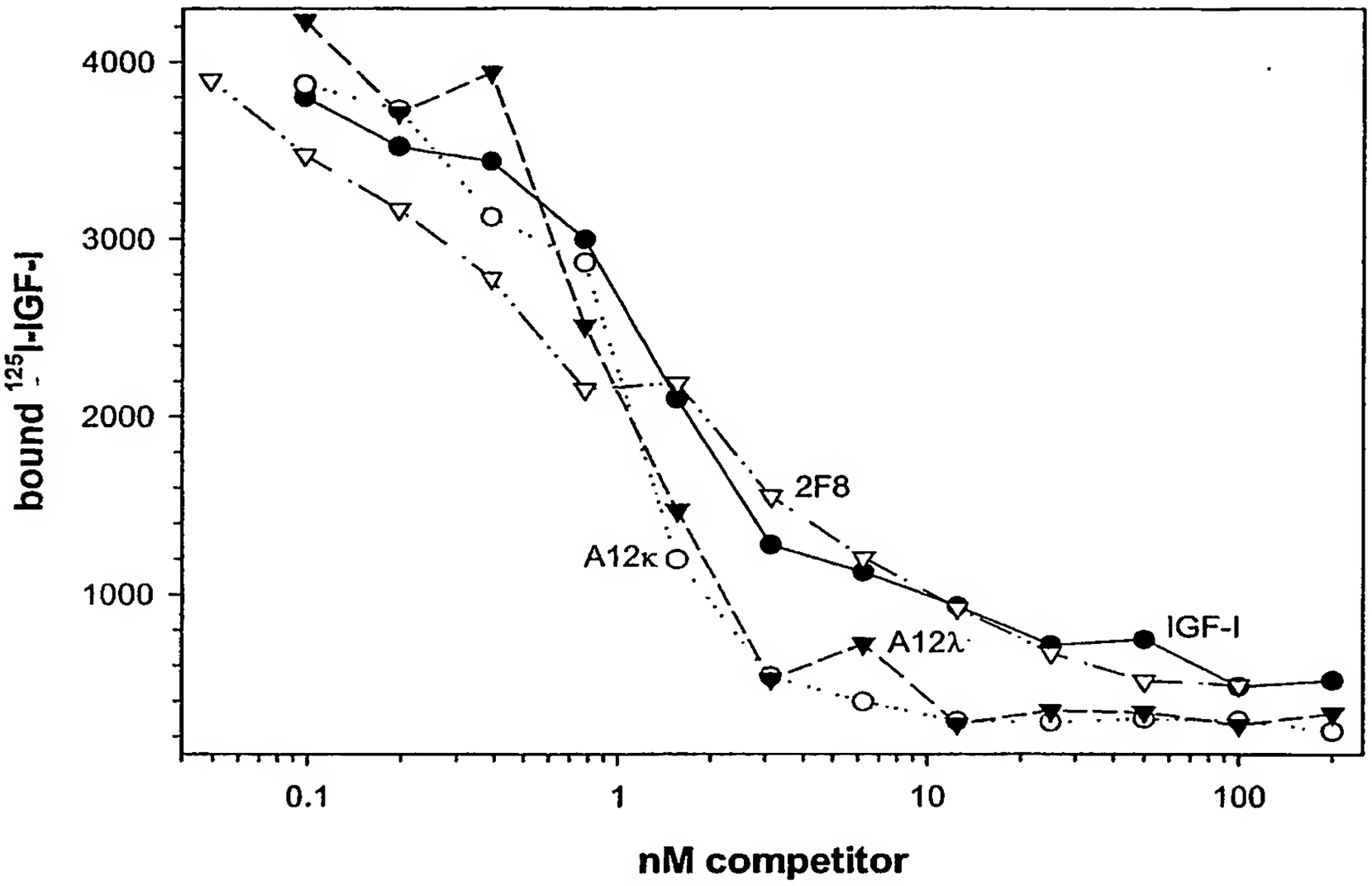

4000
3000
2000
1000
bound ^{125}I-IGF-I
2F8
A12κ
A12λ
IGF-I
0.1
1
10
100
nM competitor

Figure 16

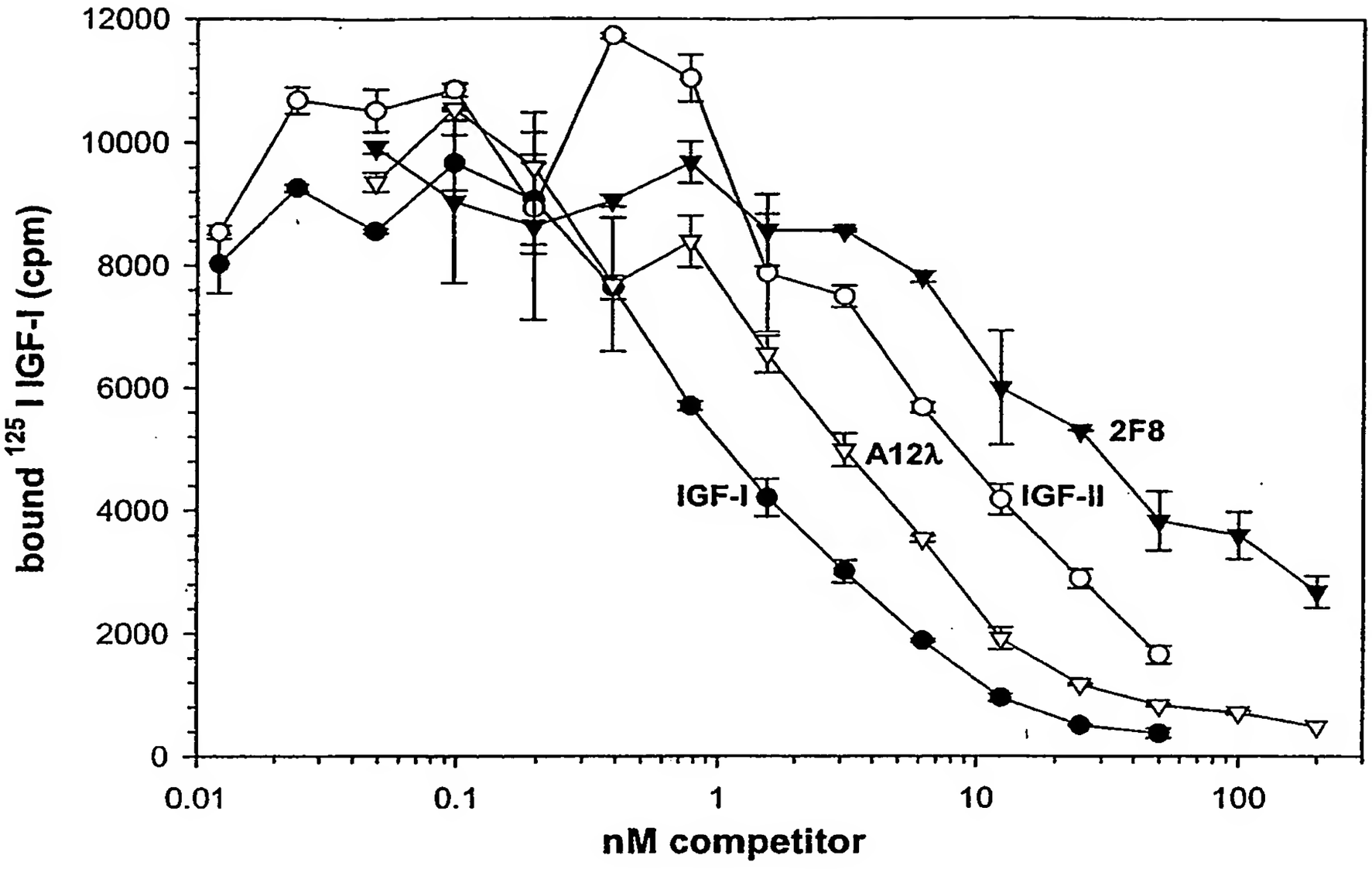

12000
10000
8000
6000
4000
2000
0
bound ^{125}I IGF-I (cpm)
0.01
0.1
1
10
100
nM competitor
IGF-I
A12λ
IGF-II
2F8

Figure 17

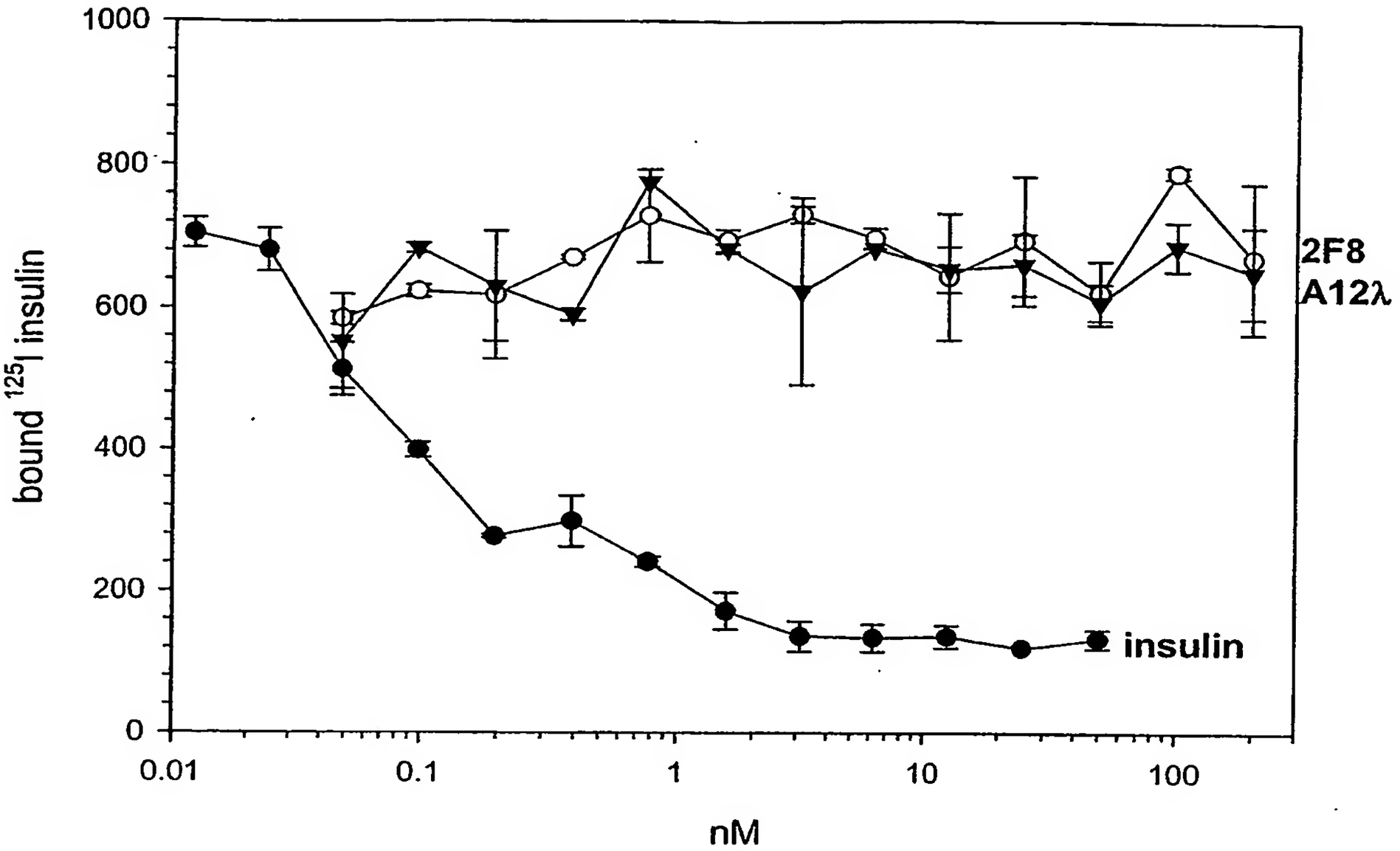

1000
800
600
400
200
0
bound ^{125}I insulin
0.01
0.1
1
10
100
nM
2F8
A12λ
insulin

Figure 18A

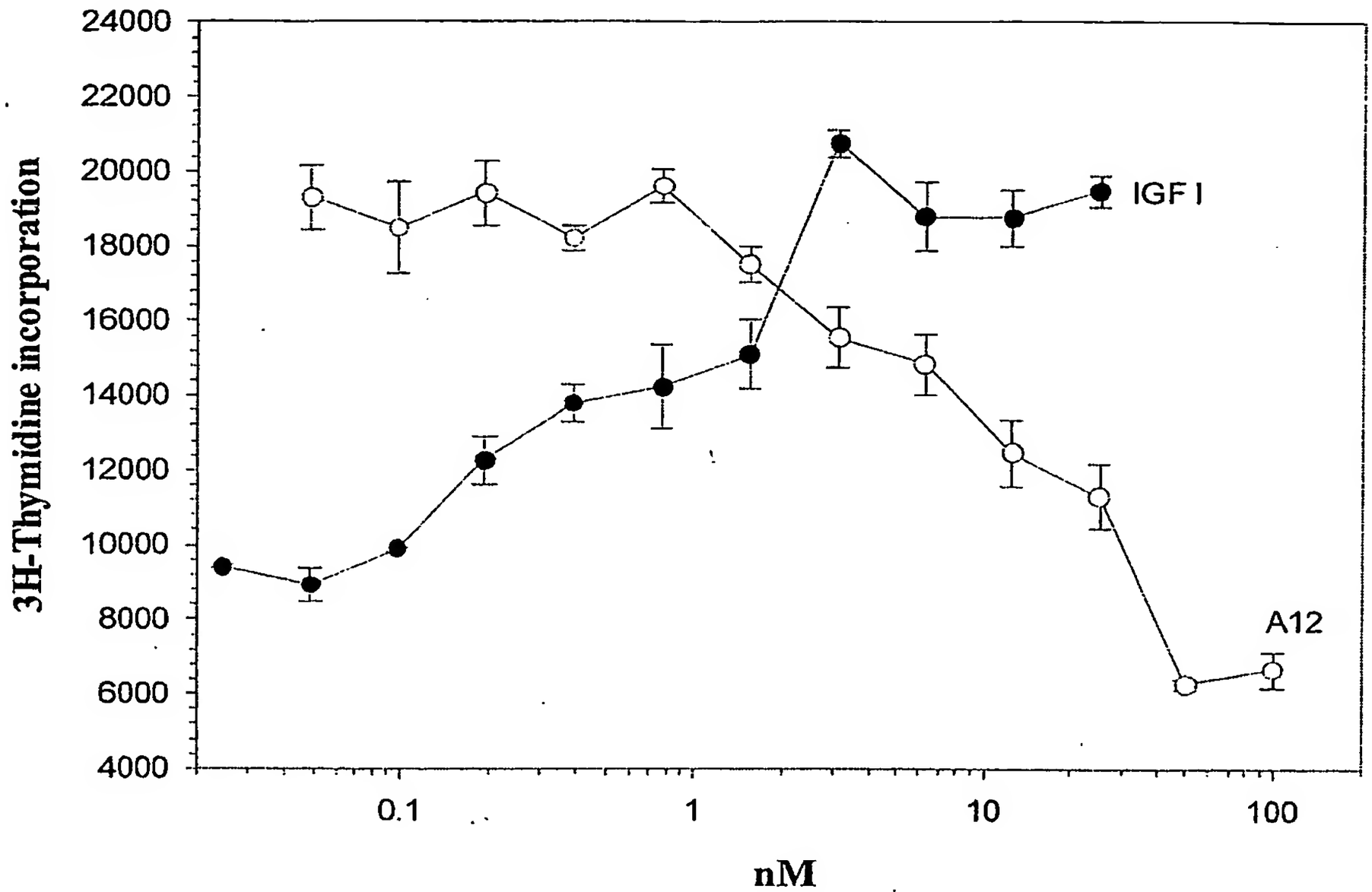

3H-Thymidine incorporation
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
0.1
1
10
100
nM
IGF I
A12

Figure 18B

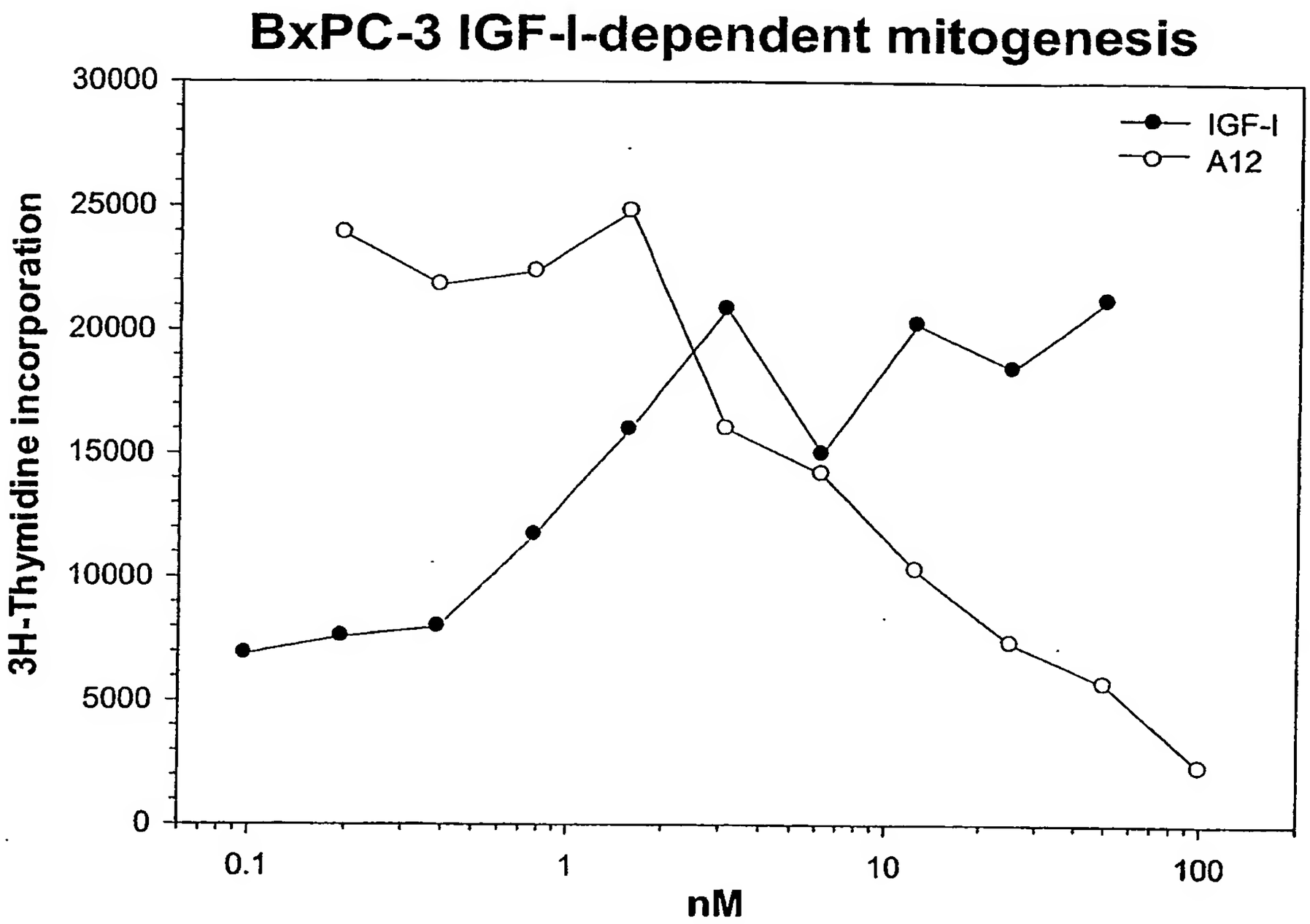

BxPC-3 IGF-I-dependent mitogenesis
3H-Thymidine incorporation
30000
25000
20000
15000
10000
5000
0
0.1
1
10
100
nM
IGF-I
A12

Figure 18C

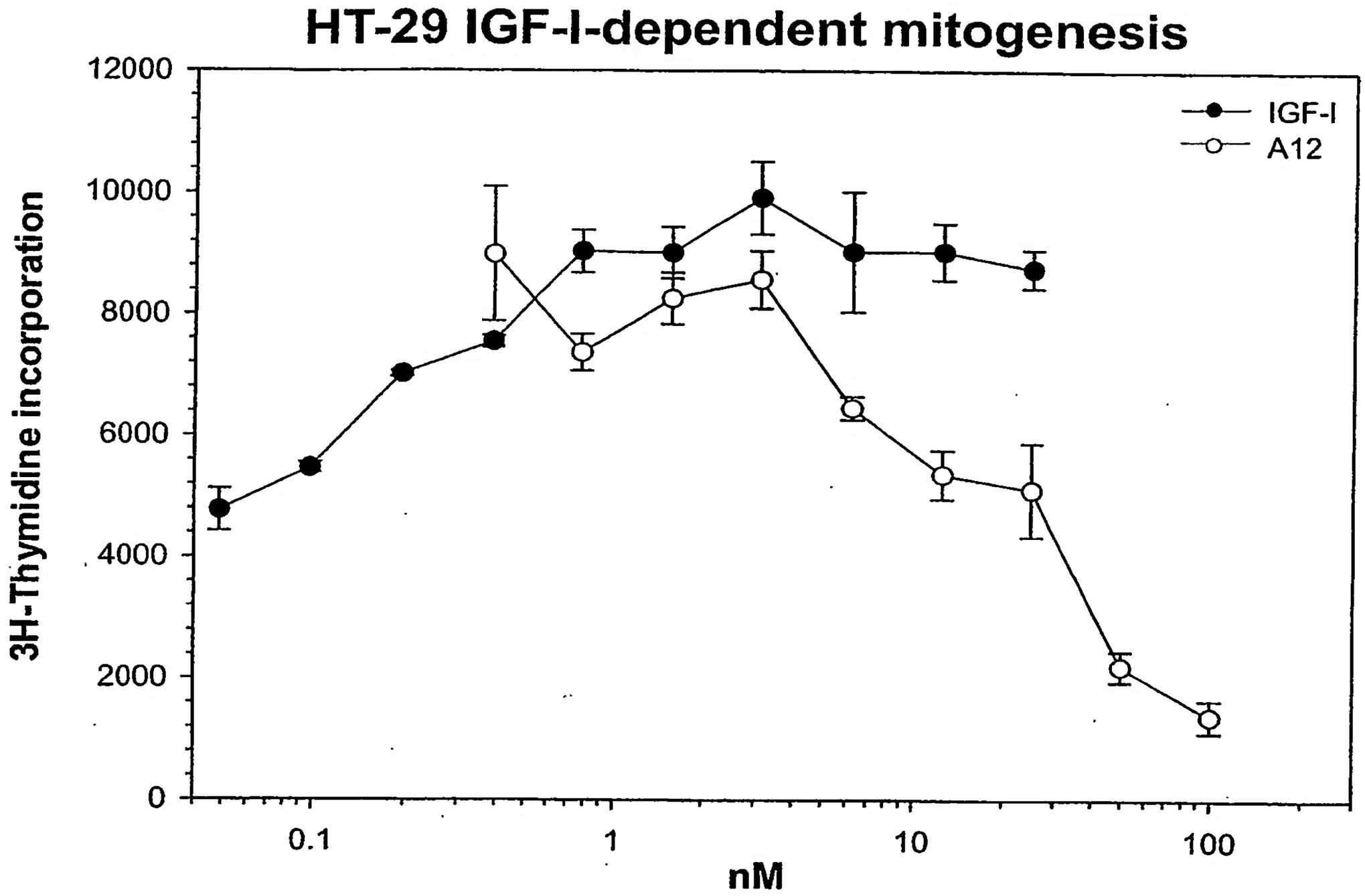

HT-29 IGF-I-dependent mitogenesis
3H-Thymidine incorporation
12000
10000
8000
6000
4000
2000
0
0.1
1
10
100
nM
IGF-I
A12

Figure 19A

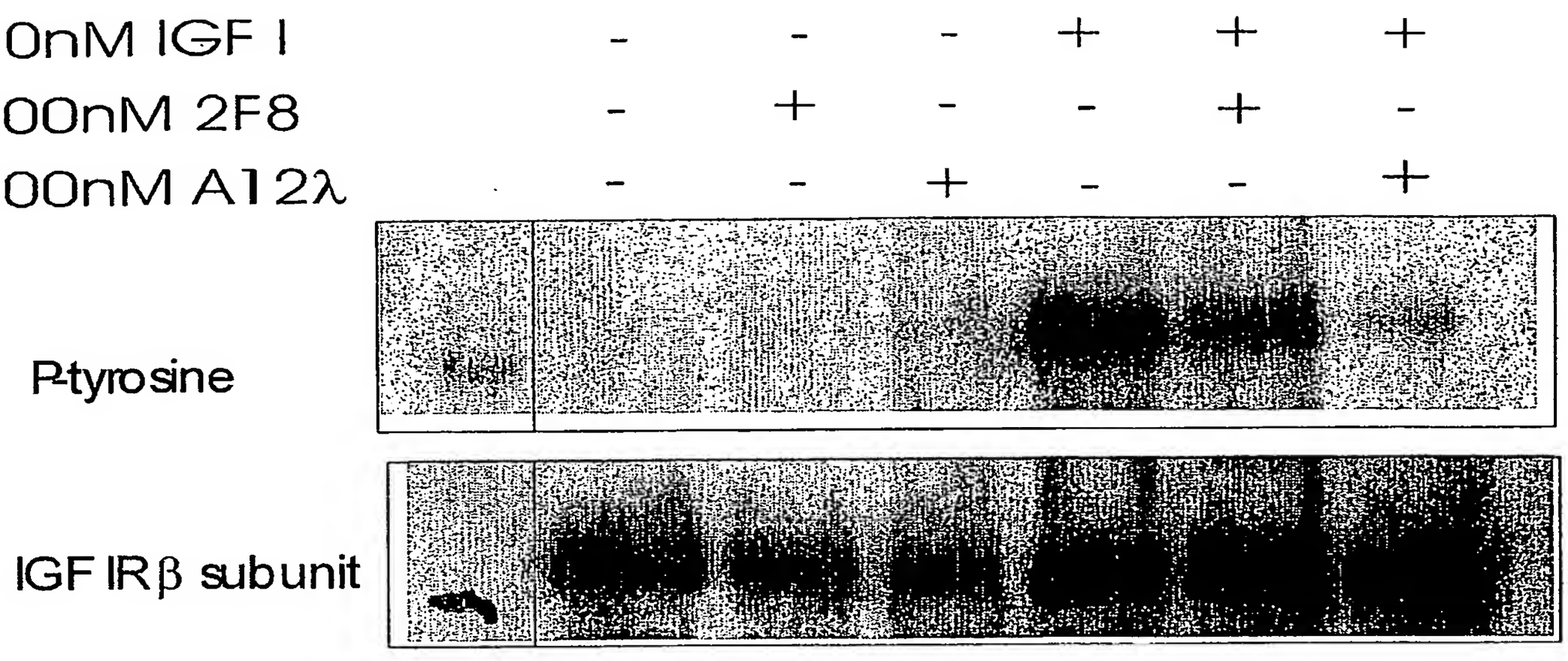


Figure 19B

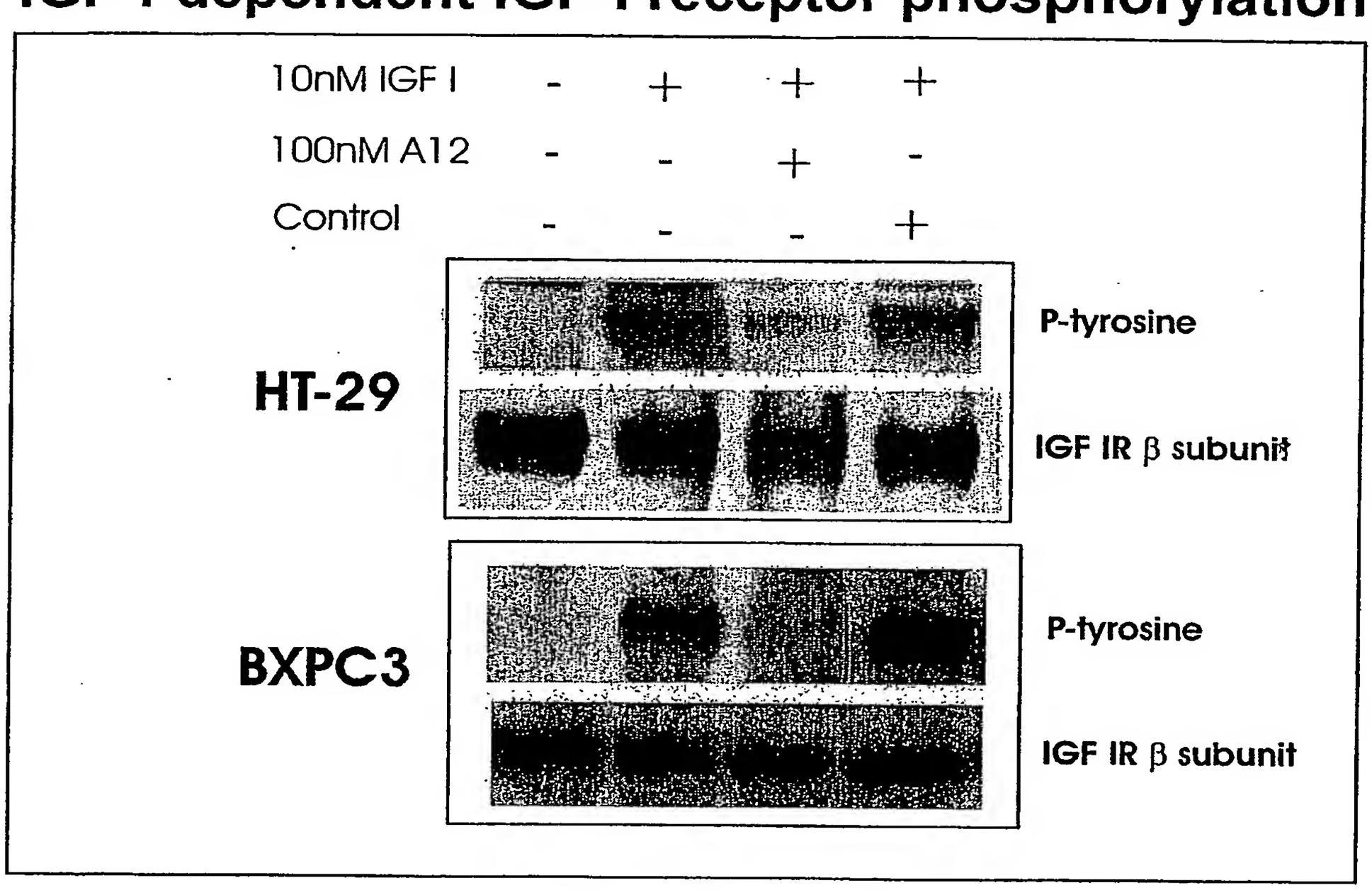
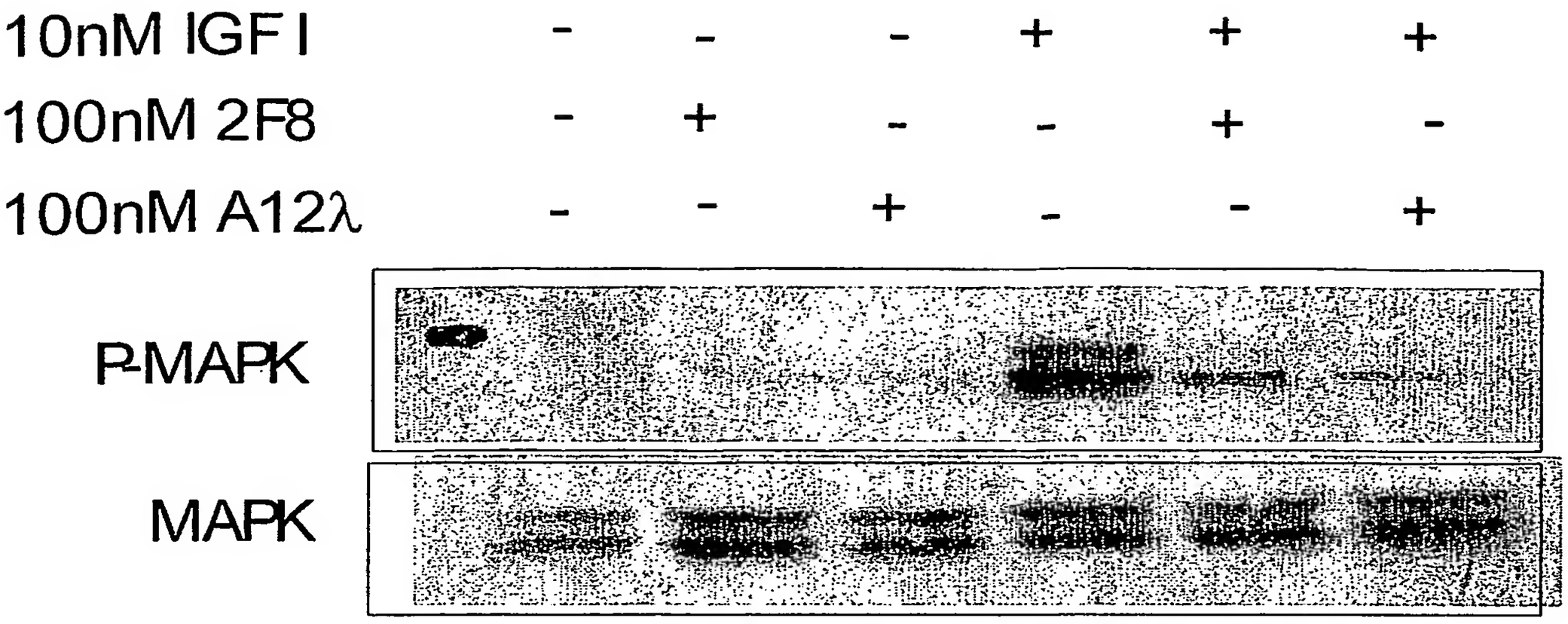

IGF-I-dependent IGF-I receptor phosphorylation
10nM IGF I - + + +
100nM A12 - - + -
Control - - - +
HT-29
P-tyrosine
IGF IR β subunit
BXPC3
P-tyrosine
IGF IR β subunit

Figure 20A



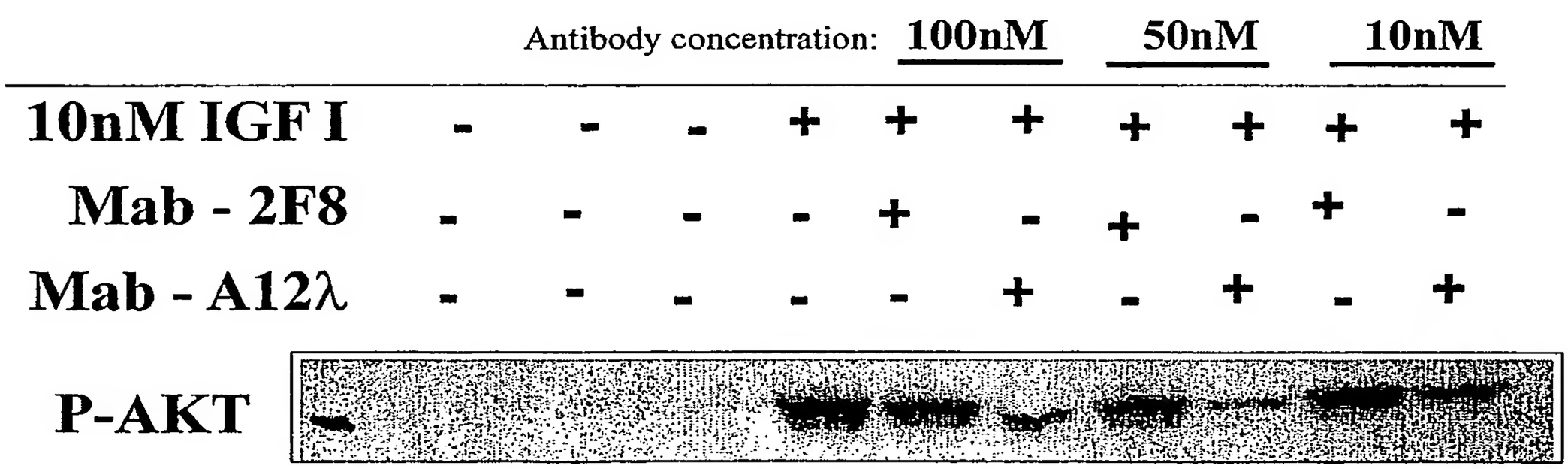
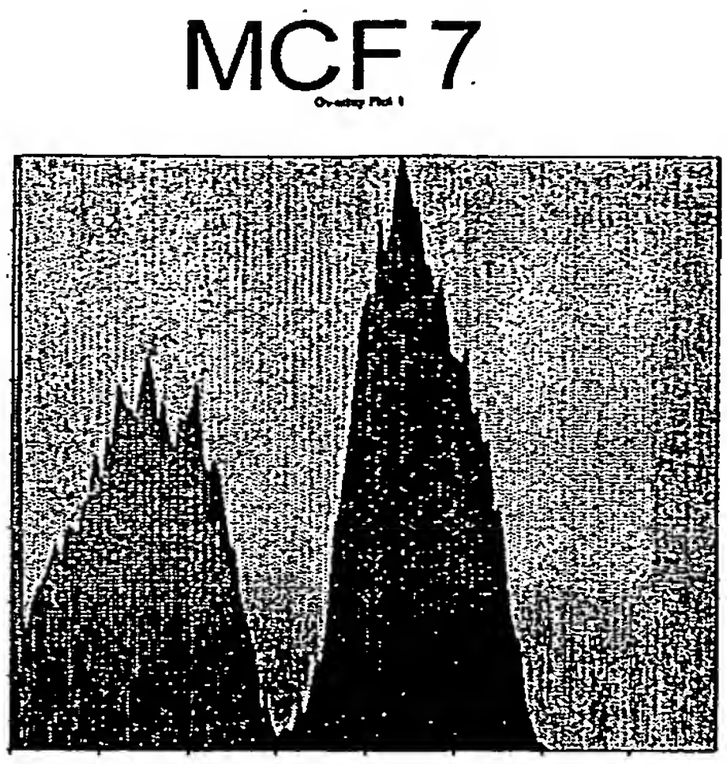
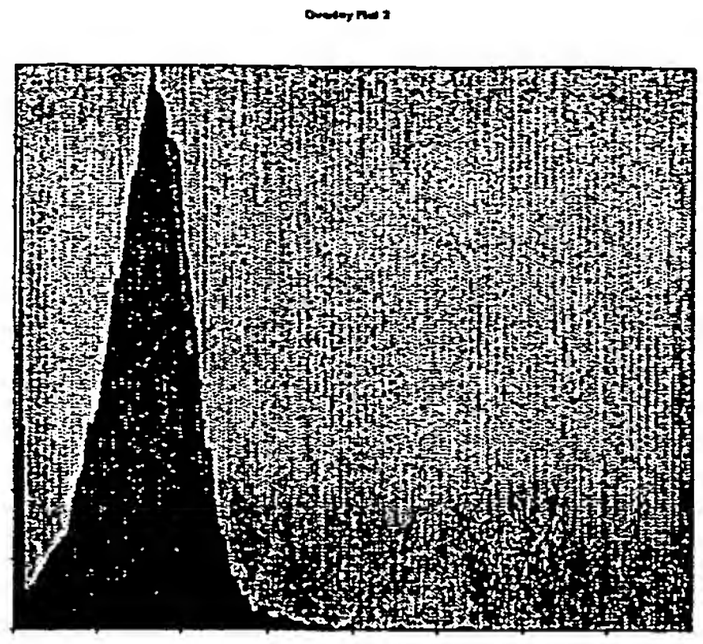
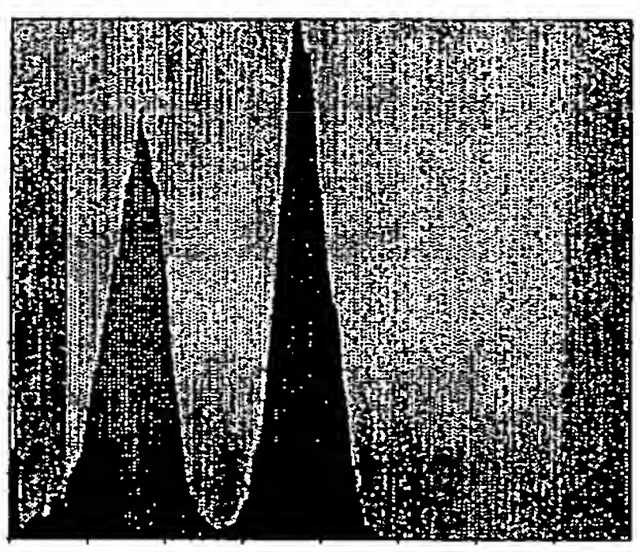


Figure 20B

Figure 21







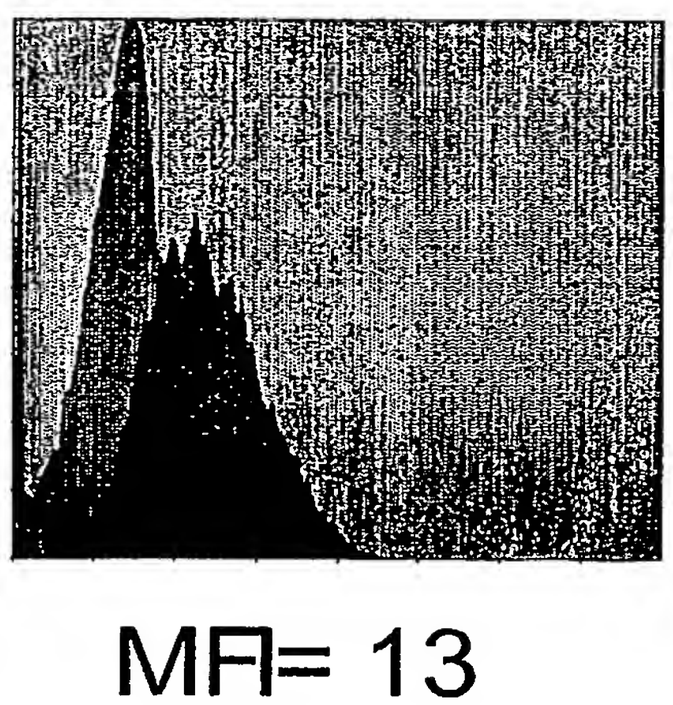


Figure 22A

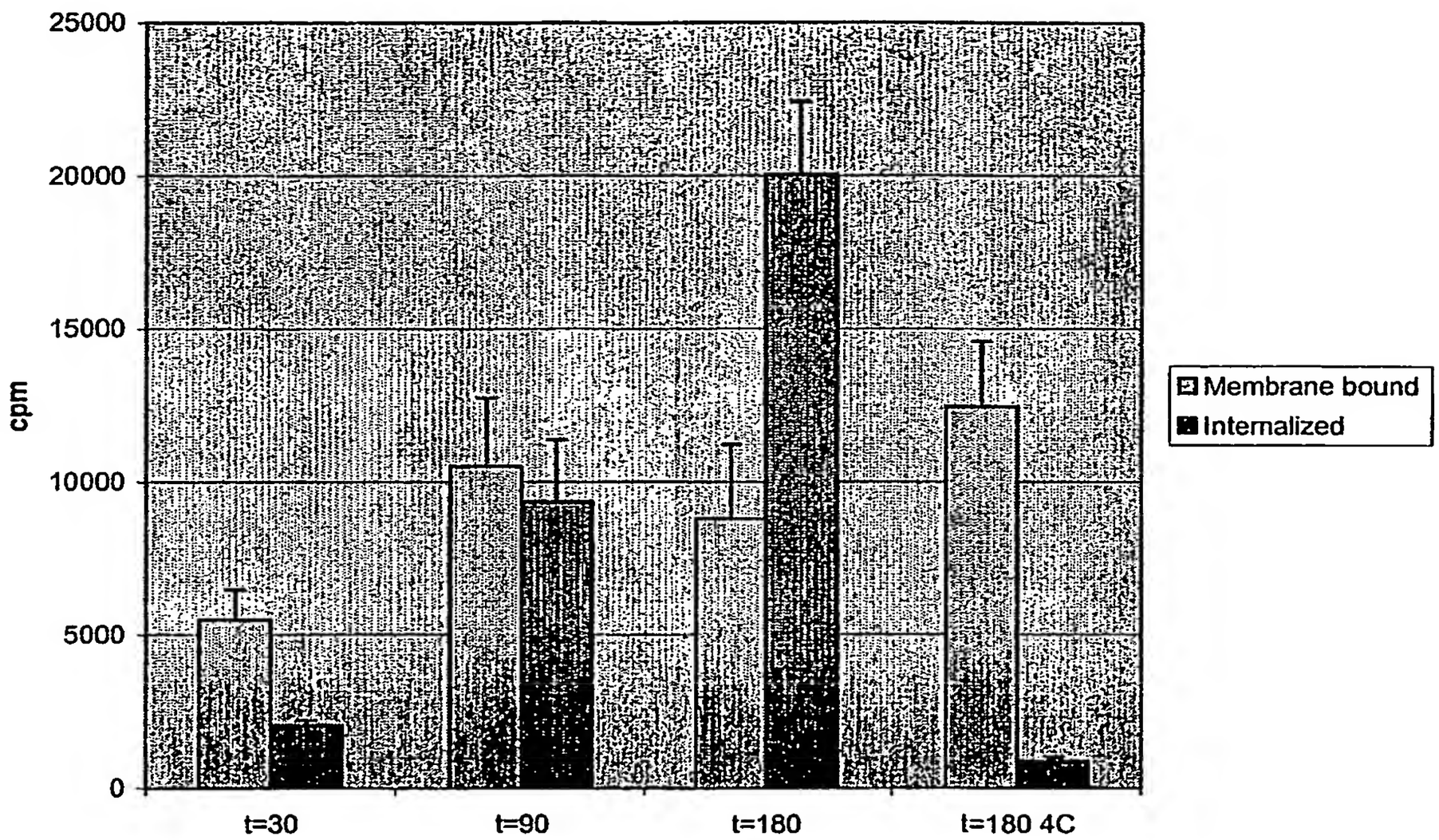

25000
20000
15000
10000
5000
0
cpm
t=30
t=90
t=180
t=180 4C
Membrane bound
Internalized

Figure 22B

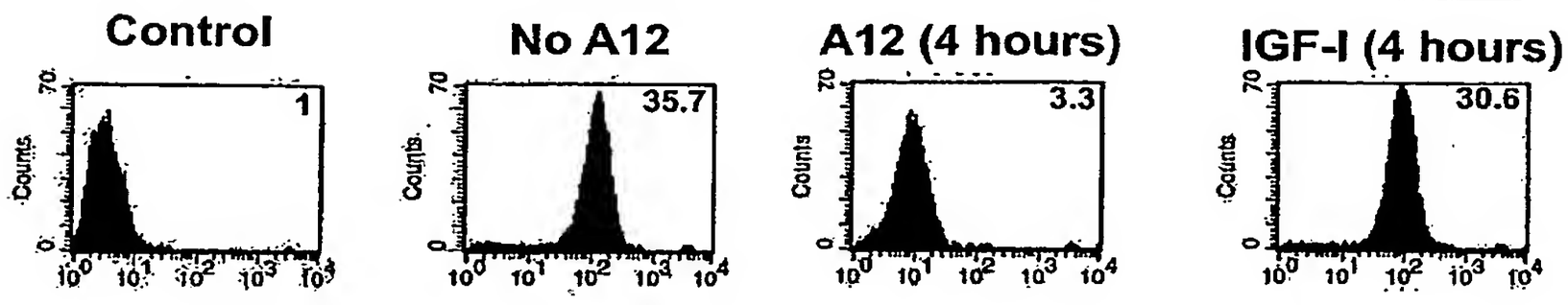

IGF-IR staining
Control
No A12
A12 (4 hours)
IGF-I (4 hours)
Counts
1
35.7
3.3
30.6

Figure 22C

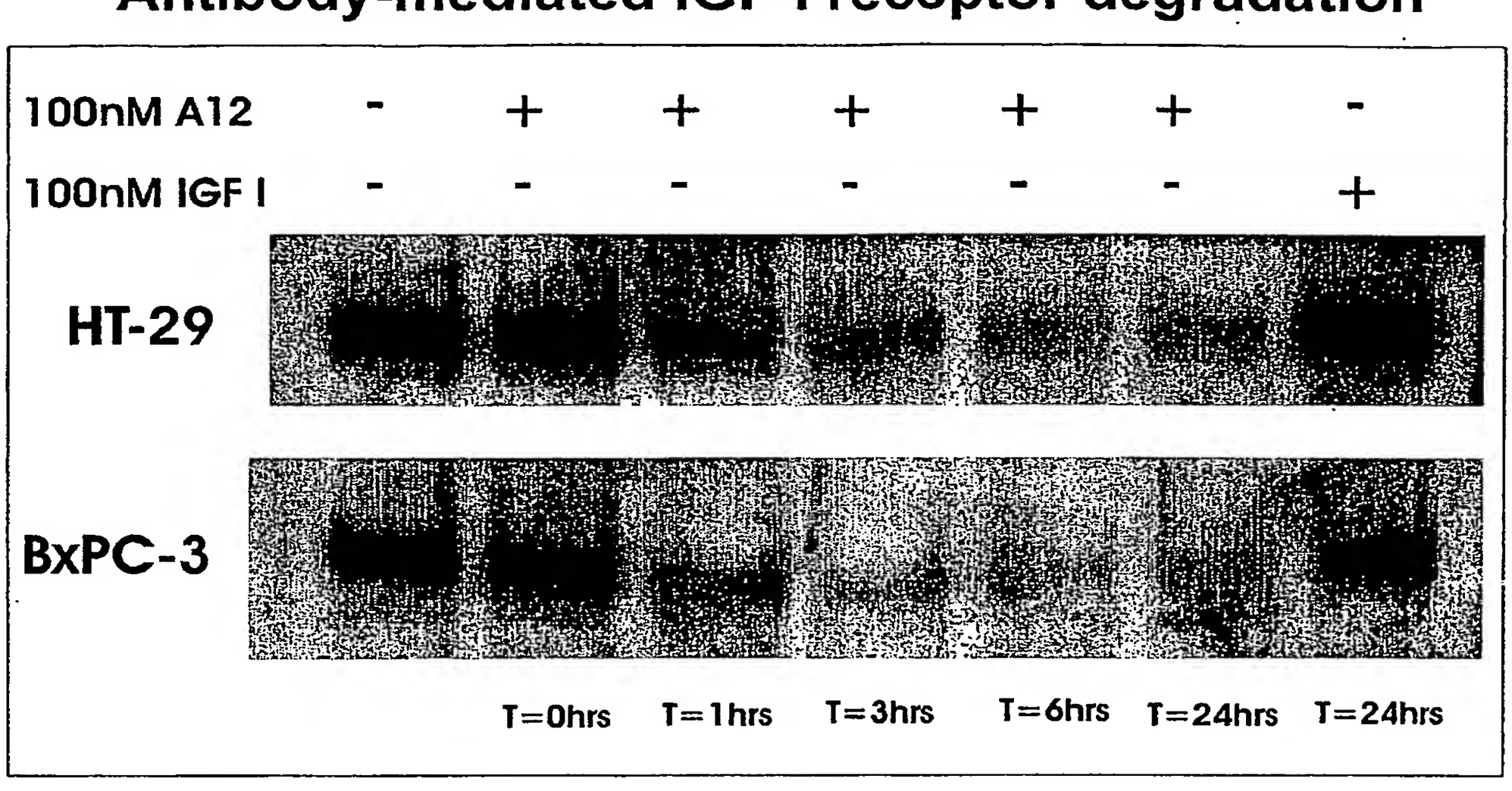

Antibody-mediated IGF-I receptor degradation
100nM A12 - + + + + + -
100nM IGF I - - - - - - +
HT-29
BxPC-3
T=0hrs T=1hrs T=3hrs T=6hrs T=24hrs T=24hrs

Figure 23

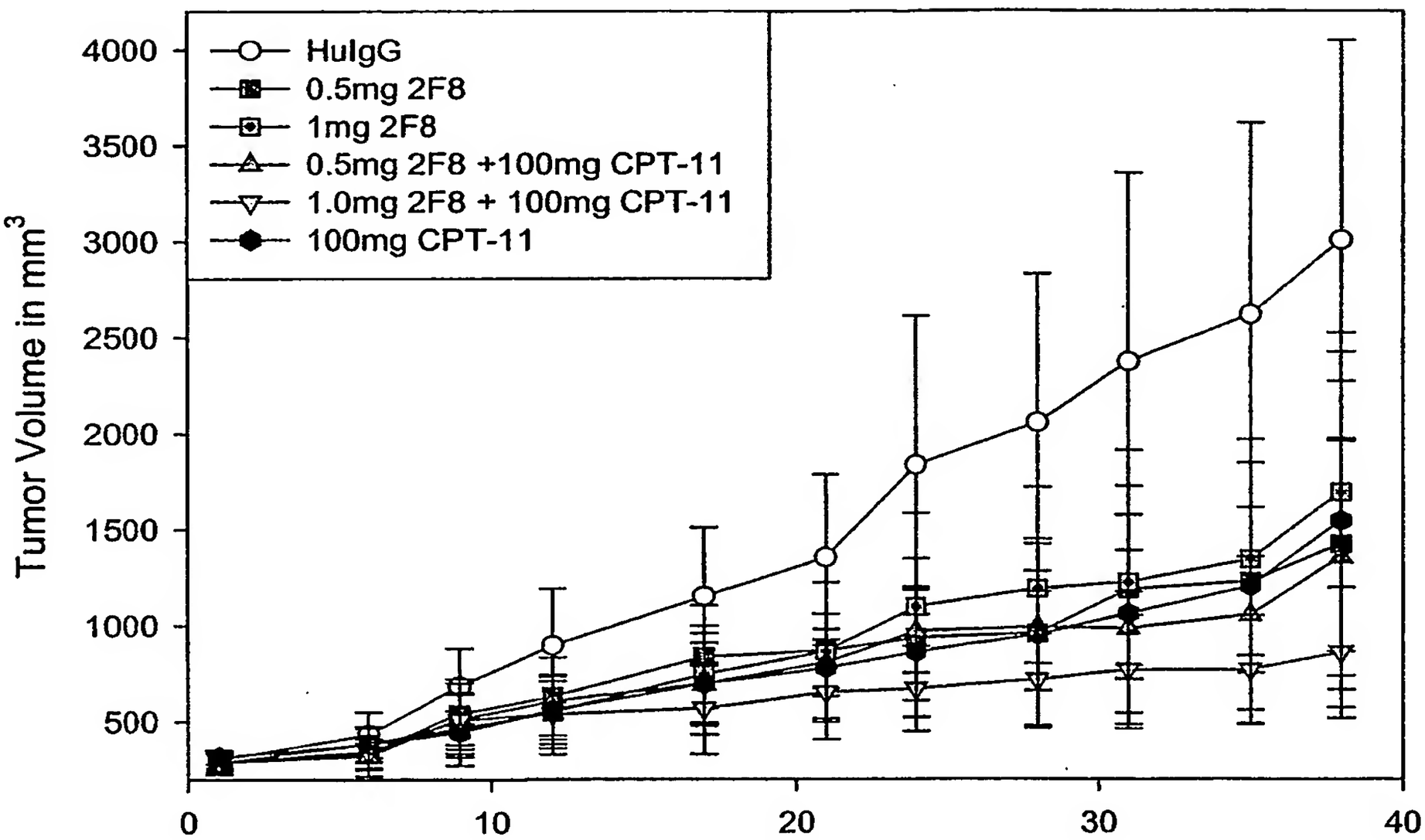

HuIgG
0.5mg 2F8
1mg 2F8
0.5mg 2F8 +100mg CPT-11
1.0mg 2F8 + 100mg CPT-11
100mg CPT-11
Tumor Volume in mm3
4000
3500
3000
2500
2000
1500
1000
500
0
10
20
30
40
Days

Figure 24

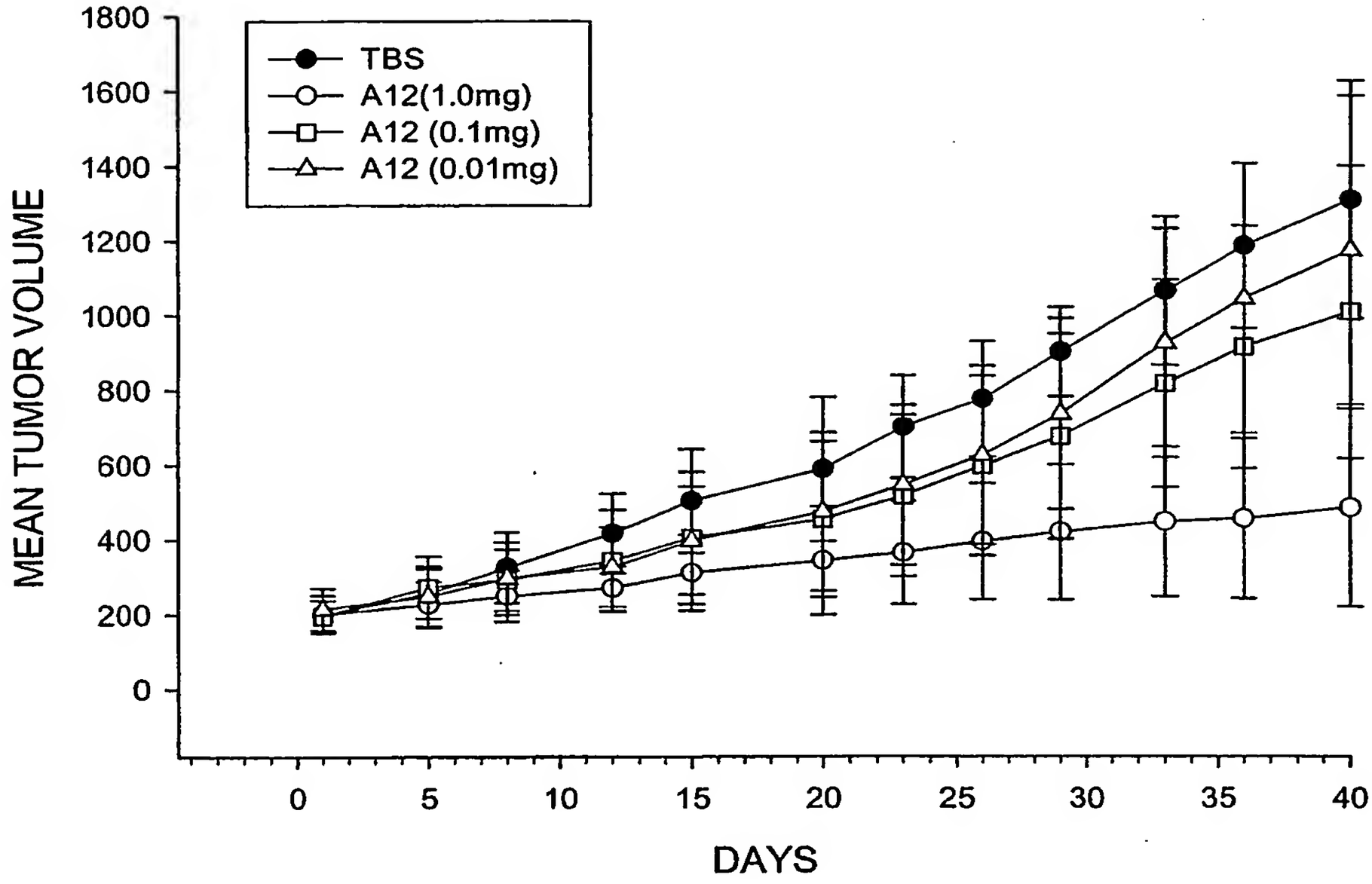

TBS
A12(1.0mg)
A12 (0.1mg)
A12 (0.01mg)
MEAN TUMOR VOLUME
1800
1600
1400
1200
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
DAYS

Figure 25:

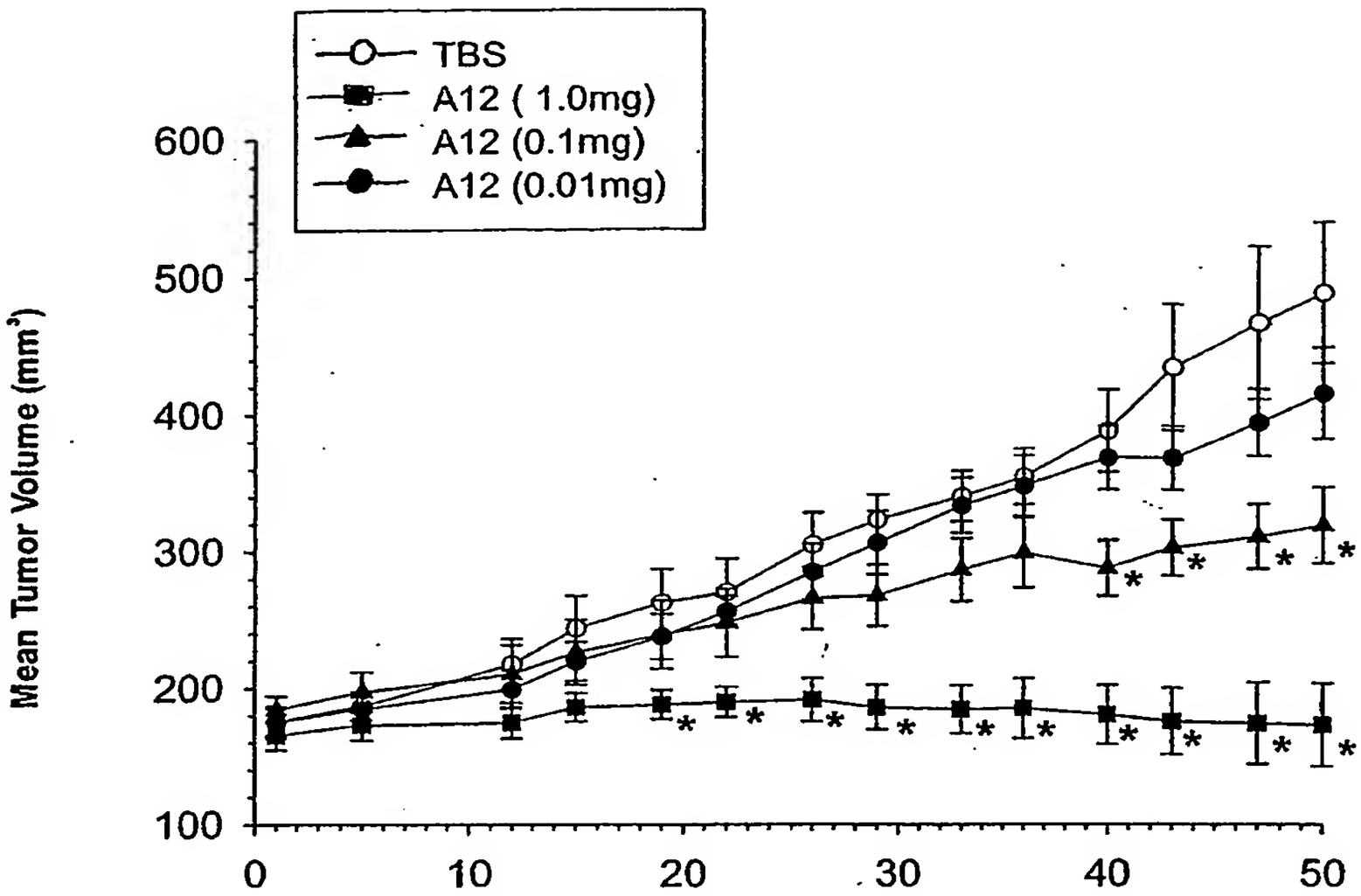

TBS
A12 (1.0mg)
A12 (0.1mg)
A12 (0.01mg)
Mean Tumor Volume (mm³)
600
500
400
300
200
100
0
10
20
30
40
50
*

Figure 26

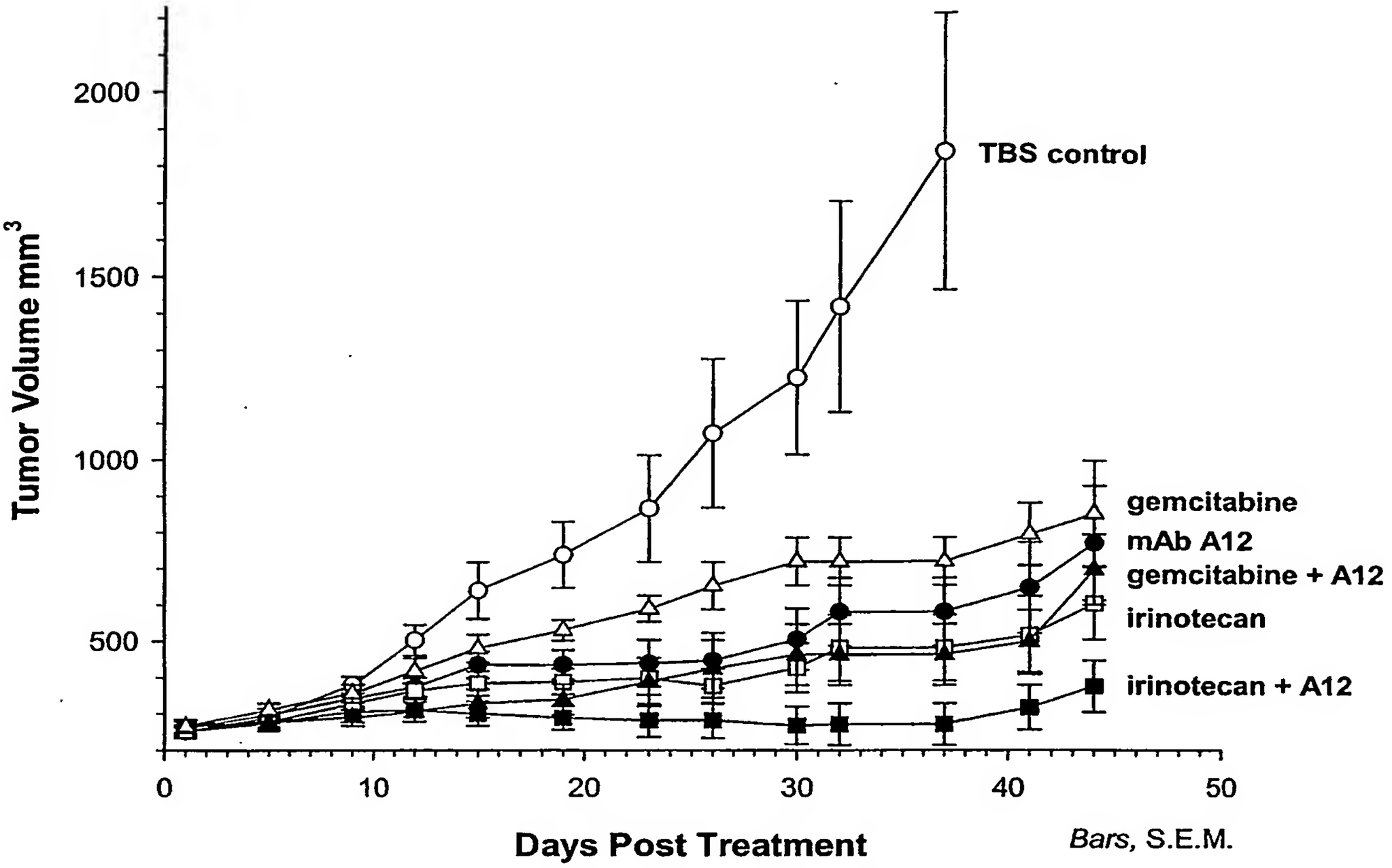
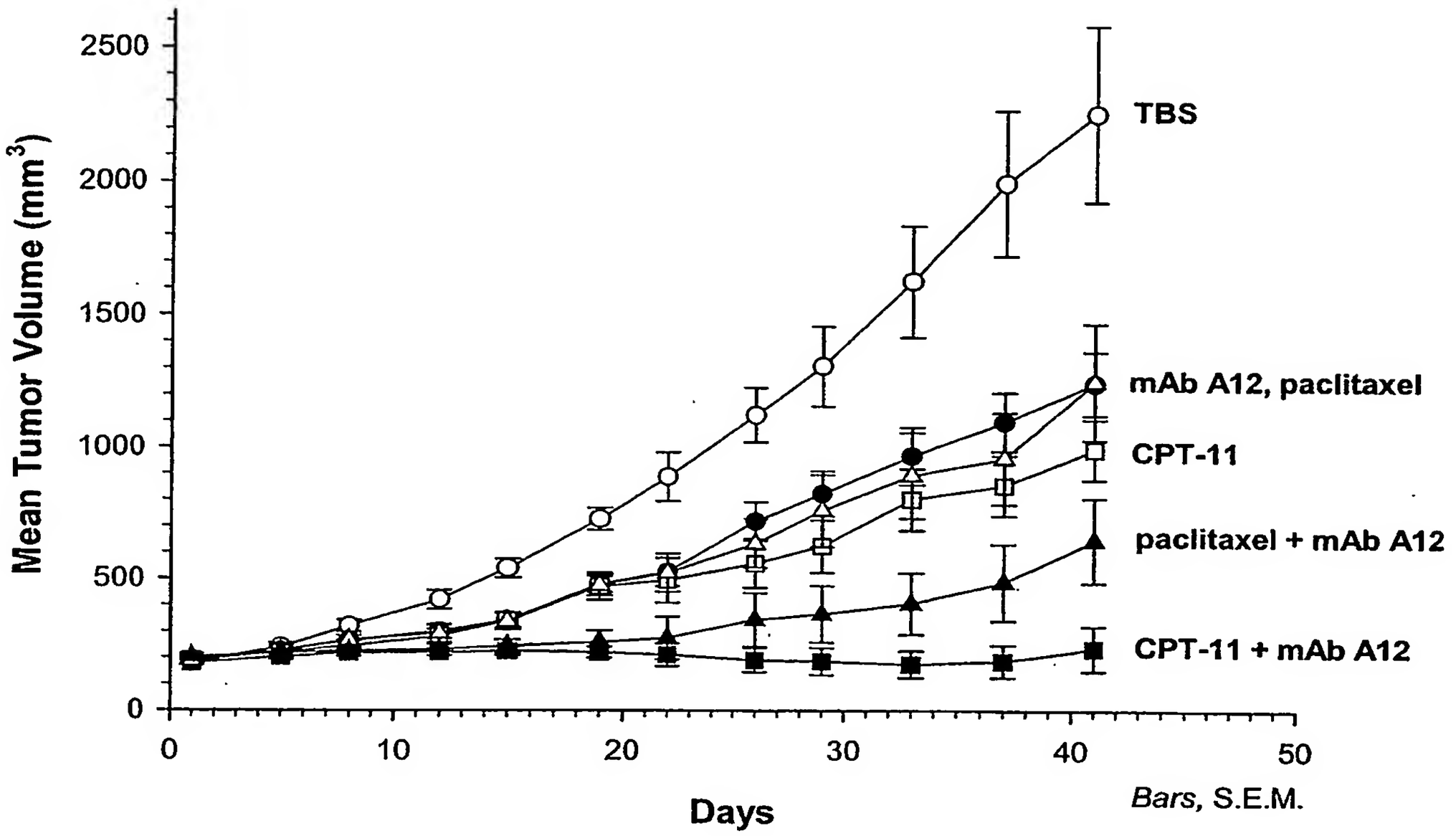

Tumor Volume mm3
2000
1500
1000
500
0
10
20
30
40
50
Days Post Treatment
TBS control
gemcitabine
mAb A12
gemcitabine + A12
irinotecan
irinotecan + A12
Bars, S.E.M.

Figure 27


Mean Tumor Volume (mm3)
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Days
TBS
mAb A12, paclitaxel
CPT-11
paclitaxel + mAb A12
CPT-11 + mAb A12
Bars, S.E.M.

SeqListing.txt

<110> ImClone Systems Incorporated
<120> Fully Human Antibodies Directed Against the Human Insulin-Like Growth Factor-1 Receptor
<130> 11245/53276
<140> To Be Assigned
<141> 2004-05-03
<150> 60/467,177
<151> 2003-05-01

<160> 33
<170> Microsoft Word 97

<210> 1
<211> 390
<212> DNA
<213> Human

<400> 1

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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
                  5                  10                  15

tcg gtg aag gtc tcc tgc aag gct tct gga ggc acc ttc agc agc tat
96
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
             20                  25                  30

gct atc agc tgg gtg cga cag gcc cct gga caa ggg ctt gag tgg atg   1
44
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
         35                  40                  45

gga ggg atc atc cct atc ttt ggt aca gca aac tac gca cag aag ttc   1
92
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
     50                  55                  60

cag ggc aga gtc acg att acc gcg gac aaa tcc acg agc aca gcc tac   2
40
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
 65                  70                  75                  80

atg gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt   2
88
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                 85                  90                  95

gcg aga gcg cca tta cga ttt ttg gag tgg tcc acc caa gac cac tac   3
36
Ala Arg Ala Pro Leu Arg Phe Leu Glu Trp Ser Thr Gln Asp His Tyr
```

100 105 110

tac tac tac tac atg gac gtc tgg ggc aaa ggg acc acg gtc acc gtc 3
84
Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val
115 120 125

3

tca agc
90
Ser Ser
130

<210> 2
<211> 130
<212> PRT
<213> Human

<400> 2

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ala Pro Leu Arg Phe Leu Glu Trp Ser Thr Gln Asp His Tyr
100 105 110

Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val
115 120 125

Ser Ser
130

<210> 3
<211> 1440
<212> DNA
<213> Human

<400> 3

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48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
5 10 15

gta cat tca gag gtc cag ctg gtg cag tct ggg gct gag gtg aag aag
96
Val His Ser Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
20 25 30

cct ggg tcc tcg gtg aag gtc tcc tgc aag gct tct gga ggc acc ttc 1
44
Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe
35 40 45

agc agc tat gct atc agc tgg gtg cga cag gcc cct gga caa ggg ctt 1
92
Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

gag tgg atg gga ggg atc atc cct atc ttt ggt aca gca aac tac gca 2
40
Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala
65 70 75 80

cag aag ttc cag ggc aga gtc acg att acc gcg gac aaa tcc acg agc 2
88
Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser
85 90 95

aca gcc tac atg gag ctg agc agc ctg aga tct gag gac acg gcc gtg 3
36
Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

tat tac tgt gcg aga gcg cca tta cga ttt ttg gag tgg tcc acc caa 3
84
Tyr Tyr Cys Ala Arg Ala Pro Leu Arg Phe Leu Glu Trp Ser Thr Gln
115 120 125

gac cac tac tac tac tac tac atg gac gtc tgg ggc aaa ggg acc acg 4
32
Asp His Tyr Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr
130 135 140

gtc acc gtc tca agc gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg 4
80
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
145 150 155 160

gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc 5
28
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
165 170 175

ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca 5
76
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
180 185 190

ggc gcc ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc 6
24
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
195 200 205

tca gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc 6
72
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
210 215 220

ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac 7
20
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
225 230 235 240

acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt gac aaa act cac 7
68
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
245 250 255

aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc 8
16
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
260 265 270

ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc 8
64
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
275 280 285

cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag 9
12
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
290 295 300

gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag 9
60
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
305 310 315 320

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08

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
325 330 335

gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag 10
56
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
340 345 350

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04
Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
355 360 365

tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc 11
52
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
370 375 380

cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc ctg 12
00
Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385 390 395 400

gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat 12
48
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
405 410 415

ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc 12
96
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
420 425 430

gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg 13
44
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
435 440 445

tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg 13
92
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
450 455 460

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35 40 45

Ser Ser Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala
65 70 75 80

Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Ala Pro Leu Arg Phe Leu Glu Trp Ser Thr Gln
115 120 125

Asp His Tyr Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr
130 135 140

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
145 150 155 160

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
165 170 175

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
180 185 190

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
195 200 205

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
210 215 220

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
225 230 235 240

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
245 250 255

Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
260 265 270

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
275 280 285

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
290 295 300

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
305 310 315 320

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
325 330 335

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
340 345 350

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
355 360 365

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
370 375 380

Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385 390 395 400

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
405 410 415

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
420 425 430

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
435 440 445

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465 470 475 479

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aca gtc agg atc aca tgc caa gga gac agc ctc aga agc tat tat gca
96
Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser Tyr Tyr Ala
20 25 30

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44
Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

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92
Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

agc tca gga aac aca gct tcc ttg acc atc act ggg gct cag gcg gaa 2
40
Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65 70 75 80

gat gag gct gac tat tac tgt aac tcc cgg gac aac agt gat aac cgt 2
88
Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Asn Ser Asp Asn Arg
85 90 95

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100 105 109

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20 25 30

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35 40 45

Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Asn Ser Asp Asn Arg
85 90 95

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100 105 109

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96
Val His Ser Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala
20 25 30

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44
Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser
35 40 45

tat tat gca agc tgg tac cag cag aag cca gga cag gcc cct gta ctt 1
92
Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu
50 55 60

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40
Val Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe
65 70 75 80

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88
Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala
85 90 95

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36
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100 105 110

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Asp Asn Arg Leu Ile Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser
115 120 125

cag ccc aag gct gcc ccc tcg gtc act ctg ttc ccg ccc tcc tct gag 432
Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
130 135 140

gag ctt caa gcc aac aag gcc aca ctg gtg tgt ctc ata agt gac ttc 480
Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
145 150 155 160

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Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
165 170 175

aag gcg gga gtg gag acc acc aca ccc tcc aaa caa agc aac aac aag 576
Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
180 185 190

tac gcg gcc agc agc tat ctg agc ctg acg cct gag cag tgg aag tcc 624
Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
195 200 205

cac aga agc tac agc tgc cag gtc acg cat gaa ggg agc acc gtg gag 672
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210 215 220

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35 40 45

Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu
50 55 60

Val Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe
65 70 75 80

Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala
85 90 95

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Asn Ser
100 105 110

Asp Asn Arg Leu Ile Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Ser
115 120 125

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
130 135 140

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
145 150 155 160

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
165 170 175

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
180 185 190

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
195 200 205

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210 215 220

Lys Thr Val Ala Pro Ala Glu Cys Ser
225 230 233

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96
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20 25 30

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44
Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Ile Leu Val Ile Tyr
35 40 45

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92
Gly Glu Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

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40
Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65 70 75 80

gat gag gct gac tac tat tgt aaa tct cgg gat ggc agt ggt caa cat 2
88
Asp Glu Ala Asp Tyr Tyr Cys Lys Ser Arg Asp Gly Ser Gly Gln His
85 90 95

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20 25 30

Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Ile Leu Val Ile Tyr
35 40 45

Gly Glu Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Lys Ser Arg Asp Gly Ser Gly Gln His
85 90 95

Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105 109

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96
Val His Ser Ser Ser Glu Leu Thr Gln Asp Pro Ala Val Ser Val Ala
20 25 30

ttg gga cag aca gtc agg atc aca tgc caa gga gac agc ctc aga agc 1
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Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser
35 40 45

tat tat gca acc tgg tac cag cag aag cca gga cag gcc cct att ctt 1
92
Tyr Tyr Ala Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Ile Leu
50 55 60

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40
Val Ile Tyr Gly Glu Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe
65 70 75 80

tct ggc tcc agc tca gga aac aca gct tcc ttg acc atc act ggg gct 2
88
Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala
85 90 95

cag gca gaa gat gag gct gac tac tat tgt aaa tct cgg gat ggc agt 3
36
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Lys Ser Arg Asp Gly Ser
100 105 110

ggt caa cat ctg gtg ttc ggc gga ggg acc aag ctg acc gtc cta ggt 384
Gly Gln His Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
115 120 125

cag ccc aag gct gcc ccc tcg gtc act ctg ttc ccg ccc tcc tct gag 432
Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
130 135 140

gag ctt caa gcc aac aag gcc aca ctg gtg tgt ctc ata agt gac ttc 480
Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
145 150 155 160

tac ccg gga gcc gtg aca gtg gcc tgg aag gca gat agc agc ccc gtc 528
Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
165 170 175

aag gcg gga gtg gag acc acc aca ccc tcc aaa caa agc aac aac aag 576
Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
180 185 190

tac gcg gcc agc agc tat ctg agc ctg acg cct gag cag tgg aag tcc 624
Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
195 200 205

cac aga agc tac agc tgc cag gtc acg cat gaa ggg agc acc gtg gag 672
His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu
210 215 220

aag aca gtg gcc cct gca gaa tgc tct tga 702
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20 25 30

Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly Asp Ser Leu Arg Ser
35 40 45

Tyr Tyr Ala Thr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Ile Leu
50 55 60

Val Ile Tyr Gly Glu Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe
65 70 75 80

Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala
85 90 95

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Lys Ser Arg Asp Gly Ser
100 105 110

Gly Gln His Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
115 120 125

Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu
130 135 140

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
145 150 155 160

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val
165 170 175

Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys
180 185 190

Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser
195 200 205

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210 215 220

Lys Thr Val Ala Pro Ala Glu Cys Ser
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<400> 17

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Tyr Tyr Met Asp Val
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<210> 23
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<210> 25
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5 10

<210> 27
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<400> 29

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